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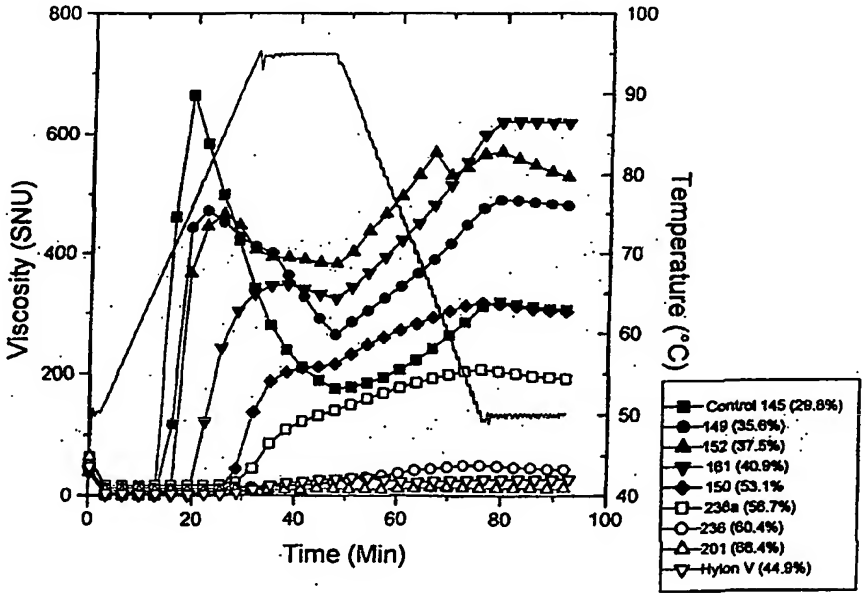

(57) Abstract

Disclosed is a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants, or a functional equivalent thereof, together with, inter alia, a corresponding polypeptide, a method of altering the characteristics of a plant, a plant having altered characteristics; and starch, particularly starch obtained from a potato plant, having novel properties.

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Title: Improvements in or Relating to Plant Starch Composition

Field of the Invention

This invention relates to novel nucleotide sequences, polypeptides encoded thereby, vectors and host cells and host organisms comprising one or more of the novel sequences, and to a method of altering one or more characteristics of an organism. The invention al;so relates to starch having novel properties and to uses thereof.

Background of the Invention

Starch is the major form of carbon reserve in plants, constituting 50% or more of the dry weight of many storage organs - e.g. tubers, seeds of cereals. Starch is used in numerous food and industrial applications. In many cases, however, it is necessary to modify the native starches, via chemical or physical means, in order to produce distinct properties to suit particular applications. It would be highly desirable to be able to produce starches with the required properties directly in the plant, thereby removing the need for additional modification. To achieve this via genetic engineering requires knowledge of the metabolic pathway of starch biosynthesis. This includes characterisation of genes and encoded gene products which catalyse the synthesis of starch. Knowledge about the regulation of starch biosynthesis raises the possibility of "re-programming" biosynthetic pathways to create starches with novel properties that could have new commercial applications.

The commercially useful properties of starch derive from the ability of the native granular form to swell and absorb water upon suitable treatment. Usually heat is required to cause granules to swell in a process known as gelatinisation, which has been defined (W A Atwell *et al*, Cereal Foods World *33*, 306-311, 1988) as "... *the collapse (disruption) of molecular orders within the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilisation. The point of initial gelatinisation and the range over which it occurs is governed by starch concentration, method of observation, granule type, and heterogeneities within the granule population under observation*". A number of techniques are available

for the determination of gelatinisation as induced by heating, a convenient and accurate method being differential scanning calorimetry, which detects the temperature range and enthalpy associated with the collapse of molecular orders within the granule. To obtain accurate and meaningful results, the peak and/or onset temperature of the endotherm observed by differential scanning calorimetry is usually determined.

The consequence of the collapse of molecular orders within starch granules is that the granules are capable of taking up water in a process known as pasting, which has been defined (W A Atwell *et al*, Cereal Foods World *33*, 306-311, 1988) as "... *the phenomenon following gelatinisation in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually, total disruption of the granules*". The best method of evaluating pasting properties is considered to be the viscoamylograph (Atwell *et al*, 1988 cited above) in which the viscosity of a stirred starch suspension is monitored under a defined time/temperature regime. A typical viscoamylograph profile for potato starch shows an initial rise in viscosity, which is considered to be due to granule swelling. In addition to the overall shape of the viscosity response in a viscoamylograph, a convenient quantitative measure is the temperature of initial viscosity development (onset). Figure 1 shows such a typical viscosity profile for potato starch, during and after cooking, and includes stages A-D which correspond to viscosity onset (A), maximum viscosity (B), complete dispersion (C) and reassociation of molecules (or retrogradation, D). In the figure, the dotted line represents viscosity (in stirring number units) of a 10% w/w starch suspension and the unbroken line shows the temperature in degrees centigrade. At a certain point, defined by the viscosity peak, granule swelling is so extensive that the resulting highly expanded structures are susceptible to mechanically-induced fragmentation under the stirring conditions used. With increased heating and holding at 95°C, further reduction in viscosity is observed due to increased fragmentation of swollen granules. This general profile has previously always been found for native potato starch.

After heating starches in water to 95°C and holding at that temperature (for typically 15 minutes), subsequent cooling to 50°C results in an increase in viscosity due to the process of retrogradation or set-back. Retrogradation (or set-back) is defined (Atwell et al.. 1988

cited above) as "*...a process which occurs when the molecules comprising gelatinised starch begin to reassociate in an ordered structure...*". At 50°C, it is primarily the amylose component which reassociates, as indicated by the increase in viscoamylograph viscosity for starch from normal maize (21.6% amylose) compared with starch from waxy maize (1.1% amylose) as shown in Figure 2. Figure 2 is a viscoamylograph of 10%w/w starch suspensions from waxy maize (solid line), conventional maize (dots and dashes), high amylose variety (hylon 5, dotted line) and a very high amylose variety (hylon 7, crosses). The temperature profile is also shown by a solid line, as in Figure 1. The extent of viscosity increase in the viscoamylograph on cooling and holding at 50°C depends on the amount of amylose which is able to reassociate due to its exudation from starch granules during the gelatinisation and pasting processes. A characteristic of amylose-rich starches from maize plants is that very little amylose is exuded from granules by gelatinisation and pasting up to 95°C, probably due to the restricted swelling of the granules. This is illustrated in Figure 2 which shows low viscosities for a high amylose (44.9%) starch (Hylon 5) from maize during gelatinisation and pasting at 95°C and little increase in viscosity on cooling and holding at 50°C. This effect is more extreme for a higher amylose content (58%, as in Hylon 7), which shows even lower viscosities in the viscoamylograph test (Figure 2). For commercially-available high amylose starches (currently available from maize plants, such as those described above), processing at greater than 100°C is usually necessary in order to generate the benefits of high amylose contents with respect to increased rates and strengths of reassociation, but use of such high temperatures is energetically unfavourable and costly. Accordingly, there is an unmet need for starches of high amylose content which can be processed below 100°C and still show enhanced levels of reassociation, as indicated for example by viscoamylograph measurements.

The properties of potato starch are useful in a variety of both food and non-food (paper, textiles, adhesives etc.) applications. However, for many applications, properties are not optimum and various chemical and physical modifications well known in the art are undertaken in order to improve useful properties. Two types of property manipulation which would be of use are: the controlled alteration of gelatinisation and pasting temperatures; and starches which suffer less granular fragmentation during pasting than

conventional starches.

Currently the only ways of manipulating the gelatinisation and pasting temperatures of potato starch are by the inclusion of additives such as sugars, polyhydroxy compounds of salts (Evans & Haisman, Starke *34*, 224-231. 1982) or by extensive physical or chemical pre-treatments (e.g. Stute, Starke *44*, 205-214, 1992). The reduction of granule fragmentation during pasting can be achieved either by extensive physical pretreatments (Stute, Starke *44*, 205-214, 1992) or by chemical cross-linking. Such processes are inconvenient and inefficient. It is therefore desirable to obtain plants which produce starch which intrinsically possesses such advantageous properties.

Starch consists of two main polysaccharides, amylose and amylopectin. Amylose is a generally linear polymer containing α-1,4 linked glucose units, while amylopectin is a highly branched polymer consisting of a α-1,4 linked glucan backbone with α-1,6 linked glucan branches. In most plant storage reserves amylopectin constitutes about 75% of the starch content. Amylopectin is synthesized by the concerted action of soluble starch synthase and starch branching enzyme [α-1,4 glucan: α-1,4 glucan 6-glycosyltransferase, EC 2.4.1.18]. Starch branching enzyme (SBE) hydrolyses α-1,4 linkages and rejoins the cleaved glucan, via an α-1,6 linkage, to an acceptor chain to produce a branched structure. The physical properties of starch are strongly affected by the relative abundance of amylose and amylopectin, and SBE is therefore a crucial enzyme in determining both the quantity and quality of starches produced in plant systems.

In most plants studied to date e.g. maize (Boyer & Preiss, 1978 Biochem. Biophys. Res. Comm. *80*, 169-175), rice (Smyth, 1988 Plant Sci. *57*, 1-8) and pea (Smith, Planta *175*, 270-279), two forms of SBE have been identified, each encoded by a separate gene. A recent review by Burton *et al.*, (1995 The Plant Journal *7*, 3-15) has demonstrated that the two forms of SBE constitute distinct classes of the enzyme such that, in general, enzymes of the same class from different plants may exhibit greater similarity than enzymes of different classes from the same plant. In their review, Burton *et al.* termed the two respective enzyme families class "A" and class "B", and the reader is referred thereto (and to the references cited therein) for a detailed discussion of the distinctions

between the two classes. One general distinction of note would appear to be the presence, in class A SBE molecules, of a flexible N-terminal domain, which is not found in class B molecules. The distinctions noted by Burton *et al.* are relied on herein to define class A and class B SBE molecules, which terms are to be interpreted accordingly.

However in potato, only one isoform of the SBE molecule (belonging to class B) has thus far been reported and only one gene cloned (Blennow & Johansson, 1991 Phytochem. *30*, 437-444, and Koßmann *et al.*, 1991 Mol. Gen. Genet. *230*, 39-44). Further, published attempts to modify the properties of starch in potato plants (by preventing expression of the single known SBE) have generally not succeeded (e.g. Müller-Rober & Koßmann 1994 Plant Cell and Environment *17*, 601-613).

Summary of the Invention

In a first aspect the invention provides a nucleotide sequence encoding an effective portion of a class A starch branching enzyme (SBE) obtainable from potato plants.

Preferably the nucleotide sequence encodes a polypeptide comprising an effective portion of the amino acid sequence shown in Figure 5 (excluding the sequence MNKRIDL, which does not represent part of the SBE molecule), or a functional equivalent thereof (which term is discussed below). The amino acid sequence shown in Figure 5 (Seq ID No. 15) includes a leader sequence which directs the polypeptide, when synthesised in potato cells, to the amyloplast. Those skilled in the art will recognise that the leader sequence is removed to produce a mature enzyme and that the leader sequence is therefore not essential for enzyme activity. Accordingly, an "effective portion" of the polypeptide is one which possesses sufficient SBE activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active when expressed in *E. coli* in the phosphorylation stimulation assay. An example of an incomplete polypeptide which nevertheless constitutes an "effective portion" is the mature enzyme lacking the leader sequence. By analogy with the pea class A SBE sequence, the potato class A sequence shown in Figure 5 probably possesses a leader sequence of about 48 amino acid residues, such that the N terminal amino acid sequence is thought to commence around the glutamic acid residue (E) at position 49 (EKSSYN... etc.). Those skilled in the art will appreciate

that an effective portion of the enzyme may well omit other parts of the sequence shown in the figure without substantial detrimental effect. For example, the C-terminal glutamic acid-rich region could be reduced in length, or possibly deleted entirely, without abolishing class A SBE activity. A comparison with other known SBE sequences, especially other class A SBE sequences (see for example, Burton *et al*, 1995 cited above), should indicate those portions which are highly conserved (and thus likely to be essential for activity) and those portions which are less well conserved (and thus are more likely to tolerate sequence changes without substantial loss of enzyme activity).

Conveniently the nucleotide sequence will comprise substantially nucleotides 289 to 2790 of the DNA sequence (Seq ID No. 14) shown in Figure 5 (which nucleotides encode the mature enzyme) or a functional equivalent thereof, and may also include further nucleotides at the 5' or 3' end. For example, for ease of expression, the sequence will desirably also comprise an in-frame ATG start codon, and may also encode a leader sequence. Thus, in one embodiment, the sequence further comprises nucleotides 145 to 288 of the sequence shown in Figure 5. Other embodiments are nucleotides 228 to 2855 of the sequence labelled "psbe2con.seq" in Figure 8, and nucleotides 57 to 2564 of the sequence shown in Figure 12 (preferably comprising an in-frame ATG start codon, such as the sequence of nucleotides 24 to 56 in the same Figure), or functional equivalents of the aforesaid sequences.

The term "functional equivalent" as applied herein to nucleotide sequences is intended to encompass those sequences which differ in their nucleotide composition to that shown in Figure 5 but which, by virtue of the degeneracy of the genetic code, encode polypeptides having identical or substantially identical amino acid sequences. It is intended that the term should also apply to sequences which are sufficiently homologous to the sequence of the invention that they can hybridise to the complement thereof under stringent hybridisation conditions - such equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably at least 95% sequence homology with the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. It will be apparent to those skilled in the art that the nucleotide sequence of the invention may also find useful application when present as an "antisense"

sequence. Accordingly, functionally equivalent sequences will also include those sequences which can hybridise, under stringent hybridisation conditions, to the sequence of the invention (rather than the complement thereof). Such "antisense" equivalents will preferably possess at least 85%, more preferably at least 90%, and most preferably 95% sequence homology with the complement of the sequence of the invention as exemplified by nucleotides 289 to 2790 of the DNA sequence shown in Figure 5. Particular functional equivalents are shown, for example, in Figures 8 and 10 (if one disregards the various frameshift mutations noted therein).

The invention also provides vectors, particularly expression vectors, comprising the nucleotide sequence of the invention. The vector will typically comprise a promoter and one or more regulatory signals of the type well known to those skilled in the art. The invention also includes provision of cells transformed (which term encompasses transduction and transfection) with a vector comprising the nucleotide sequence of the invention.

The invention further provides a class A SBE polypeptide, obtainable from potato plants. In particular the invention provides the polypeptide in substantially pure form, especially in a form free from other plant-derived (especially potato plant-derived) components, which can be readily accomplished by expression of the relevant nucleotide sequence in a suitable non-plant host (such as any one of the yeast strains routinely used for expression purposes, e.g. *Pichia spp.* or *Saccharomyces spp*). Typically the enzyme will substantially comprise the sequence of amino acid residues 49 to 882 shown in Figure 5 (disregarding the sequence MNKRIDL, which is not part of the enzyme), or a functional equivalent thereof. The polypeptide of the invention may be used in a method of modifying starch *in vitro*, comprising treating starch under suitable conditions (e.g. appropriate temperature, pH, etc) with an effective amount of the polypeptide according to the invention.

The term "functional equivalent", as applied herein to amino acid sequences, is intended to encompass amino acid sequences substantially similar to that shown in Figure 5, such that the polypeptide possesses sufficient activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active in *E. coli* in the

phosphorylation stimulation assay. Typically such functionally equivalent amino acid sequences will preferably possess at least 85%, more preferably at least 90%, and most preferably at least 95% sequence identity with the amino acid sequence of the mature enzyme (i.e. minus leader sequence) shown in Figure 5. Those skilled in the art will appreciate that conservative substitutions may be made generally throughout the molecule without substantially affecting the activity of the enzyme. Moreover, some non-conservative substitutions may be tolerated, especially in the less highly conserved regions of the molecule. Such substitutions may be made, for example, to modify slightly the activity of the enzyme. The polypeptide may, if desired, include a leader sequence, such as that exemplified by residues 1 to 48 of the amino acid sequence shown in Figure 5, although other leader sequences and signal peptides and the like are known and may be included.

A portion of the nucleotide sequence of the invention has been introduced into a plant and found to affect the characteristics of the plant. In particular, introduction of the sequence of the invention, operably linked in the antisense orientation to a suitable promoter, was found to reduce the amount of branched starch molecules in the plant. Additionally, it has recently been demonstrated in other experimental systems that "sense suppression" can also occur (i.e. expression of an introduced sequence operably linked in the sense orientation can interfere, by some unknown mechanism, with the expression of the native gene), as described by Matzke & Matzke (1995 Plant Physiol. *107*, 679-685). Any one of the methods mentioned by Matzke & Matzke could, in theory, be used to affect the expression in a host of a homologous SBE gene.

It is believed that antisense methods are mainly operable by the production of antisense mRNA which hybridises to the sense mRNA, preventing its translation into functional polypeptide, possibly by causing the hybrid RNA to be degraded (e.g. Sheehy *et al.*, 1988 PNAS *85*, 8805-8809; Van der Krol *et al.*, Mol. Gen. Genet. *220*, 204-212). Sense suppression also requires homology between the introduced sequence and the target gene, but the exact mechanism is unclear. It is apparent however that, in relation to both antisense and sense suppression, neither a full length nucleotide sequence, nor a "native" sequence is essential. Preferably the "effective portion" used in the method will comprise

at least one third of the full length sequence, but by simple trial and error other fragments (smaller or larger) may be found which are functional in altering the characteristics of the plant.

Thus, in a further aspect the invention provides a method of altering the characteristics of a plant, comprising introducing into the plant an effective portion of the sequence of the invention operably linked to a suitable promoter active in the plant. Conveniently the sequence will be linked in the anti-sense orientation to the promoter. Preferably the plant is a potato plant. Conveniently, the characteristic altered relates to the starch content and/or starch composition of the plant (i.e. amount and/or type of starch present in the plant). Preferably the method of altering the characteristics of the plant will also comprise the introduction of one or more further sequences, in addition to an effective portion of the sequence of the invention. The introduced sequence of the invention and the one or more further sequences (which may be sense or antisense sequences) may be operably linked to a single promoter (which would ensure both sequences were transcribed at essentially the same time), or may be operably linked to separate promoters (which may be necessary for optimal expression). Where separate promoters are employed they may be identical to each other or different. Suitable promoters are well known to those skilled in the art and include both constitutive and inducible types. Examples include the CaMV 35S promoter (e.g. single or tandem repeat) and the patatin promoter. Advantageously the promoter will be tissue-specific. Desirably the promoter will cause expression of the operably linked sequence at substantial levels only in the tissue of the plant where starch synthesis and/or starch storage mainly occurs. Thus, for example, where the sequence is introduced into a potato plant, the operably linked promoter may be tuber-specific, such as the patatin promoter.

Desirably, for example, the method will also comprise the introduction of an effective portion of a sequence encoding a class B SBE, operably linked in the antisense orientation to a suitable promoter active in the plant. Desirably the further sequence will comprise an effective portion of the sequence encoding the potato class B SBE molecule. Conveniently the further sequence will comprise an effective portion of the sequence described by Blennow & Johansson (1991 Phytochem. *30*, 437-444) or that disclosed in

WO92/11375. More preferably, the further sequence will comprise at least an effective portion of the sequence disclosed in International Patent Application No. WO 95/26407. Use of antisense sequences against both class A and class B SBE in combination has now been found by the present inventors to result in the production of starch having very greatly altered properties (see below). Those skilled in the art will appreciate the possibility that, if the plant already comprises a sense or antisense sequence which efficiently inhibits the class B SBE activity, introduction of a sense or antisense sequence to inhibit class A SBE activity (thereby producing a plant with inhibition of both class A and class B activity) might alter greatly the properties of the starch in the plant, without the need for introduction of one or more further sequences. Thus the sequence of the invention is conveniently introduced into plants already having low levels of class A and/or class B SBE activity, such that the inhibition resulting from the introduction of the sequence of the invention is likely to have a more pronounced effect.

The sequence of the invention, and the one or more further sequences if desired, can be introduced into the plant by any one of a number of well-known techniques (e.g. Agrobacterium-mediated transformation, or by "biolistic" methods). The sequences are likely to be most effective in inhibiting SBE activity in potato plants, but theoretically could be introduced into any plant. Desirable examples include pea, tomato, maize, wheat, rice, barley, sweet potato and cassava plants. Preferably the plant will comprise a natural gene encoding an SBE molecule which exhibits reasonable homology with the introduced nucleic acid sequence of the invention.

In another aspect, the invention provides a plant cell, or a plant or the progeny thereof, which has been altered by the method defined above. The progeny of the altered plant may be obtained, for example, by vegetative propagation, or by crossing the altered plant and reserving the seed so obtained. The invention also provides parts of the altered plant, such as storage organs. Conveniently, for example, the invention provides tubers comprising altered starch, said tubers being obtained from an altered plant or the progeny thereof. Potato tubers obtained from altered plants (or the progeny thereof) will be particularly useful materials in certain industrial applications and for the preparation and/or processing of foodstuffs and may be used, for example, to prepare low-fat waffles and

chips (amylose generally being used as a coating to prevent fat uptake), and to prepare mashed potato (especially "instant" mashed potato) having particular characteristics.

In particular relation to potato plants, the invention provides a potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant. The plant may have been altered by the method defined above, or may have been selected by conventional breeding to be deleted for the class A SBE gene, presence or absence of which can be readily determined by screening samples of the plants with a nucleic acid probe or antibody specific for the potato class A gene or gene product respectively.

The invention also provides starch extracted from a plant altered by the method defined above, or the progeny of such a plant, the starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. The invention further provides a method of making altered starch, comprising altering a plant by the method defined above and extracting therefrom starch having altered properties compared to starch extracted from equivalent, but unaltered, plants. Use of nucleotide sequences in accordance with the invention has allowed the present inventors to produce potato starches having a wide variety of novel properties.

In particular the invention provides the following: a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated endotherm peak temperature as judged by DSC, compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated viscosity onset temperature (conveniently elevated by 10 - 25°C) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased peak viscosity (conveniently decreased by 240 - 700SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method

defined above, containing starch which, when extracted from the plant, has an increased pasting viscosity (conveniently increased by 37 - 260SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an increased set-back viscosity (conveniently increased by 224 - 313 SNUs) as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has a decreased set-back viscosity as judged by viscoamylograph compared to starch extracted from a similar, but unaltered, plant; and a plant (especially a potato plant) altered by the method defined above, containing starch which, when extracted from the plant, has an elevated amylose content as judged by iodometric assay (i.e. by the method of Morrison & Laignelet 1983, cited above) compared to starch extracted from a similar, but unaltered, plant. The invention also provides for starch obtainable or obtained from such plants as aforesaid.

In particular the invention provides for starch which, as extracted from a potato plant by wet milling at ambient temperature, has one or more of the following properties, as judged by viscoamylograph analysis performed according to the conditions defined below: viscosity onset temperature in the range 70-95°C (preferably 75-95°C); peak viscosity in the range 500 - 12 stirring number units; pasting viscosity in the range 214 - 434 stirring number units; set-back viscosity in the range 450 - 618 or 14 - 192 stirring number units; or displays no significant increase in viscosity during viscoamylograph. Peak, pasting and set-back viscosities are defined below. Viscosity onset temperature is the temperature at which there is a sudden, marked increase in viscosity from baseline levels during viscoamylograph, and is a term well-known to those skilled in the art.

In other particular embodiments, the invention provides starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNUs and a set-back viscosity in the range 275-618 SNUs as judged by viscoamylograph according to the protocol defined below; and starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding

phase (step 5) and has a set-back viscosity of 303 SNUs or less as judged by viscoamylograph according to the protocol defined below.

For the purposes of the present invention, viscoamylograph conditions are understood to pertain to analysis of a 10% (w/w) aqueous suspension of starch at atmospheric pressure, using a Newport Scientific Rapid Visco Analyser with a heating profile of: holding at 50°C for 2 minutes (step 1), heating from 50 to 95°C at a rate of 1.5°C per minute (step 2), holding at 95°C for 15 minutes (step 3), cooling from 95 to 50°C at a rate of 1.5°C per minute (step 4), and then holding at 50°C for 15 minutes (step 5). Peak viscosity may be defined for present purposes as the maximum viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

In yet another aspect the invention provides starch from a potato plant having an apparent amylose content (% w/w) of at least 35%, as judged by iodometric assay according to the method described by Morrison & Laignelet (1983 J. Cereal Science *1*, 9-20). Preferably the starch will have an amylose content of at least 40%, more preferably at least 50%, and most preferably at least 66%. Starch obtained directly from a potato plant and having such properties has not hitherto been produced. Indeed, as a result of the present invention, it is now possible to generate *in vivo* potato starch which has some properties analogous to the very high amylose starches (e.g. Hylon 7) obtainable from maize.

Starches with high (at least 35%) amylose contents find commercial application as, amongst other reasons, the amylose component of starch reassociates more strongly and rapidly than the amylopectin component during retrogradation processes. This may result, for example, in pastes with higher viscosities, gels of greater cohesion, or films of greater strength for starches with high (at least 35%) compared with normal (less than 35%) amylose contents. Alternatively, starches may be obtained with very high amylose contents, such that the granule structure is substantially preserved during heating, resulting in starch suspensions which demonstrate substantially no increase in viscosity during

cooking (i.e. there is no significant viscosity increase during viscoamylograph conditions defined above). Such starches typically exhibit a viscosity increase of less than 10% (preferably less than 5%) during viscoamylograph under the conditions defined above.

In commerce, these valuable properties are currently obtained from starches of high amylose content derived from maize plants. It would be of commercial value to have an alternative source of high amylose starches from potato as other characteristics such as granule size, organoleptic properties and textural qualities may distinguish application performances of high amylose starches from maize and potato plants.

Thus high amylose starch obtained by the method of the present invention may find application in many different technological fields, which may be broadly categorised into two groups: food products and processing; and "Industrial" applications. Under the heading of food products, the novel starches of the present invention may find application as, for example, films, barriers, coatings or gelling agents. In general, high amylose content starches absorb less fat during frying than starches with low amylose content, thus the high amylose content starches of the invention may be advantageously used in preparing low fat fried products (e.g. potato chips, crisps and the like). The novel starches may also be employed with advantage in preparing confectionery and in granular and retrograded "resistant" starches. "Resistant" starch is starch which is resistant to digestion by α-amylase. As such, resistant starch is not digested by α-amylases present in the human small intestine, but passes into the colon where it exhibits properties similar to soluble and insoluble dietary fibre. Resistant starch is thus of great benefit in foodstuffs due to its low calorific value and its high dietary fibre content. Resistant starch is formed by the retrogradation (akin to recrystallization) of amylose from starch gels. Such retrogradation is inhibited by amylopectin. Accordingly, the high amylose starches of the present invention are excellent starting materials for the preparation of resistant starch. Suitable methods for the preparation of resistant starch are well-known to those skilled in the art and include, for example, those described in US 5,051,271 and US 5,281,276. Conveniently the resistant starches provided by the present invention comprise at least 5% total dietary fibre, as judged by the method of Prosky *et al.*, (1985 J. Assoc. Off. Anal. Chem. *68*, 677), mentioned in US 5,281, 276.

Under the heading of "Industrial" applications, the novel starches of the invention may be advantageously employed, for example, in corrugating adhesives, in biodegradable products such as loose fill packaging and foamed shapes, and in the production of glass fibers and textiles.

Those skilled in the art will appreciate that the novel starches of the invention may, if desired, be subjected *in vitro* to conventional enzymatic, physical and/or chemical modification, such as cross-linking, introduction of hydrophobic groups (e.g. octenyl succinic acid, dodecyl succinic acid), or derivatization (e.g. by means of esterification or etherification).

In yet another aspect the invention provides high (35% or more) amylose starches which generate paste viscosities greater than those obtained from high amylose starches from maize plants after processing at temperatures below 100°C. This provides the advantage of more economical starch gelatinisation and pasting treatments through the use of lower processing temperatures than are currently required for high amylose starches from maize plants.

The invention will now be further described by way of illustrative example and with reference to the drawings, of which:

Figure 1 shows a typical viscoamylograph for a 10% w/w suspension of potato starch;

Figure 2 shows vsicoamylographs for 10% suspensions of starch from various maize varieties;

Figure 3 is a schematic representation of the cloning strategy used by the present inventors;

Figure 4a shows the amino acid alignment of the C-terminal portion of starch branching enzyme isoforms from various sources: amino acid residues matching the consensus

sequence are shaded;

Figure 4b shows the alignment of DNA sequences of various starch branching enzyme isoforms which encode a conserved amino acid sequence;

Figure 5 shows the DNA sequence (Seq ID No. 14) and predicted amino acid sequence (Seq ID No. 15) of a full length potato class A SBE cDNA clone obtained by PCR;

Figure 6 shows a comparison of the most highly conserved part of the amino acid sequences of potato class A (uppermost sequence) and class B (lowermost sequence) SBE molecules;

Figure 7 shows a comparison of the amino acid sequence of the full length potato class A (uppermost sequence) and pea (lowermost sequence) class A SBE molecules;

Figure 8 shows a DNA alignment of various full length potato class A SBE clones obtained by the inventors;

Figure 9 shows the DNA sequence of a potato class A SBE clone determined by direct sequencing of PCR products, together with the predicted amino acid sequence;

Figure 10 is a multiple DNA alignment of various full length potato SBE A clones obtained by the inventors;

Figure 11 is a schematic illustration of the plasmid pSJ64;

Figure 12 shows the DNA sequence and predicted amino acid sequence of the full length potato class A SBE clone as present in the plasmid pSJ90; and

Figure 13 shows viscoamylographs for 10% w/w suspensions of starch from various transgenic potato plants made by the relevant method aspect of the invention.

Examples

Example 1

Cloning of Potato class A SBE

The strategy for cloning the second form of starch branching enzyme from potato is shown in Figure 3. The small arrowheads represent primers used by the inventors in PCR and RACE protocols. The approximate size of the fragments isolated is indicated by the numerals on the right of the Figure. By way of explanation, a comparison of the amino acid sequences of several cloned plant starch branching enzymes (SBE) from maize (class A), pea (class A), maize (class B), rice (class B) and potato (class B), as well as human glycogen branching enzyme, allowed the inventors to identify a region in the carboxy-terminal one third of the protein which is almost completely conserved (GYLNFMGNEFGHPEWIDFPR) (Figure 4a). A multiple alignment of the DNA sequences (human, pea class A, potato class B, maize class B, maize class A and rice class B, respectively) corresponding to this region is shown in Figure 4b and was used to design an oligo which would potentially hybridize to all known plant starch branching enzymes: AATTT(C/T)ATGGGIAA(C/T)GA(A/G)TT(C/T)GG (Seq ID No. 20).

Library PCR

The initial isolation of a partial potato class A SBE cDNA clone was from an amplified potato tuber cDNA library in the λZap vector (Stratagene). One half μL of a potato cDNA library (titre 2.3 x 10^9pfu/mL) was used as template in a 50 μL reaction containing 100 pmol of a 16 fold degenerate POTSBE primer and 25 pmol of a T7 primer (present in the λZap vector 3' to the cDNA sequences - see Figure 3), 100 μM dNTPs, 2.5 U Taq polymerase and the buffer supplied with the Taq polymerase (Stratagene). All components except the enzyme were added to a 0.5 mL microcentrifuge tube, covered with mineral oil and incubated at 94°C for 7 minutes and then held at 55°C, while the Taq polymerase was added and mixed by pipetting. PCR was then performed by incubating for 1 min at 94°C, 1 min at 58°C and 3 minutes at 72°C, for 35 cycles. The PCR products were extracted with phenol/chloroform, ethanol precipitated and resuspended in TE pH 8.0 before cloning into the T/A cloning vector pT7BlueR (Invitrogen).

Several fragments between 600 and 1300 bp were amplified. These were isolated from an agarose gel and cloned into the pT7BlueR T/A cloning vector. Restriction mapping of 24 randomly selected clones showed that they belonged to several different groups (based on size and presence/absence of restriction sites). Initially four clones were chosen for sequencing. Of these four, two were found to correspond to the known potato class B SBE sequence, however the other two, although homologous, differed significantly and were more similar to the pea class A SBE sequence, suggesting that they belonged to the class A family of branching enzymes (Burton *et al.*, 1995 The Plant Journal, cited above). The latter two clones (~ 800bp) were sequenced fully. They both contained at the 5' end the sequence corresponding to the degenerate oligonucleotide used in the PCR and had a predicted open reading frame of 192 amino acids. The deduced amino acid sequence was highly homologous to that of the pea class A SBE.

The ~800 bp PCR derived cDNA fragment (corresponding to nucleotides 2281 to 3076 of the psbe2 con.seq sequence shown in Figure 8) was used as a probe to screen the potato tuber cDNA library. From one hundred and eighty thousand plaques, seven positives were obtained in the primary screen. PCR analysis showed that five of these clones were smaller than the original 800 bp cDNA clone, so these were not analysed further. The two other clones (designated 3.2.1 and 3.1.1) were approximately 1200 and 1500 bp in length respectively. These were sequenced from their 5' ends and the combined consensus sequence aligned with the sequence from the PCR generated clones. The cDNA clone 3.2.1 was excised from the phage vector and plasmid DNA was prepared and the insert fully sequenced. Several attempts to obtain longer clones from the library were unsuccessful, therefore clones containing the 5' end of the full length gene were obtained using RACE (rapid amplification of cDNA ends).

Rapid Amplification of cDNA ends (RACE) and PCR conditions

RACE was performed essentially according to Frohman (1992 Amplifications 11-15). Two μg of total RNA from mature potato tubers was heated to 65°C for 5 min and quick cooled on ice. The RNA was then reverse transcribed in a 20 μL reaction for 1 hour at 37°C using BRL's M-MLV reverse transcriptase and buffer with 1 mM DTT, 1 mM dNTPs, 1 U/μL RNAsin (Promega) and 500 pmol random hexamers (Pharmacia) as

primer. Excess primers were removed on a Centricon 100 column and cDNA was recovered and precipitated with isopropanol. cDNA was A-tailed in a volume of 20 μl using 10 units terminal transferase (BRL), 200 μM dATP for 10 min at 37°C, followed by 5 min at 65°C. The reaction was then diluted to 0.5 ml with TE pH 8 and stored at 4°C as the cDNA pool. cDNA clones were isolated by PCR amplification using the primers $R_oR_idT_{17}$, R_o and POTSBE24. The PCR was performed in 50 μL using a hot start technique: 10 μL of the cDNA pool was heated to 94°C in water for 5 min with 25 pmol POTSBE24, 25 pmol R_o and 2.5 pmol of $R_oR_idT_{17}$ and cooled to 75°C. Five μL of 10 x PCR buffer (Stratagene), 200 μM dNTPs and 1.25 units of Taq polymerase were added, the mixture heated at 45°C for 2 min and 72°C for 40 min followed by 35 cycles of 94°C for 45 sec, 50°C for 25 sec, 72°C for 1.5 min and a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1% low melting agarose gels and the smear covering the range 600-800 bp fragments was excised and used in a second PCR amplification with 25 pmol of R_i and POTSBE25 primers in a 50 μL reaction (28 cycles of 94°C for 1 min, 50°C 1 min, 72°C 2 min). Products were purified by chloroform extraction and cloned into pT7 Blue. PCR was used to screen the colonies and the longest clones were sequenced.

The first round of RACE only extended the length of the SBE sequence approximately 100 bases, therefore a new A-tailed cDNA library was constructed using the class A SBE specific oligo POTSBE24 (10 pmol) in an attempt to recover longer RACE products. The first and second round PCR reactions were performed using new class A SBE primers (POTSBE 28 and 29 respectively) derived from the new sequence data. Conditions were as before except that the elongation step in the first PCR was for 3 min and the second PCR consisted of 28 cycles at 94 °C for 45 seconds, 55 °C for 25 sec and 72 °C for 1 min 45 sec.

Clones ranging in size from 400 bp to 1.4 kb were isolated and sequenced. The combined sequence of the longest RACE products and cDNA clones predicted a full length gene of about 3150 nucleotides, excluding the poly(A) tail (psbe 2con.seq in Fig. 8).

As the sequence of the 5' half of the gene was compiled from the sequence of several

RACE products generated using Taq polymerase, it was possible that the compiled sequence did not represent that of a single mRNA species and/or had nucleotide sequence changes. The 5' 1600 bases of the gene was therefore re-isolated by PCR using Ultma, a thermostable DNA polymerase which, because it possesses a 3'-5' exonuclease activity, has a lower error rate compared to Taq polymerase. Several PCR products were cloned and restriction mapped and found to differ in the number of *Hind* III, *Ssp* I, and *Eco*R I sites. These differences do not represent PCR artefacts as they were observed in clones obtained from independent PCR reactions (data not shown) and indicate that there are several forms of the class A SBE gene transcribed in potato tubers.

In order to ensure that the sequence of the full length cDNA clone was derived from a single mRNA species it was therefore necessary to PCR the entire gene in one piece. cDNA was prepared according to the RACE protocol except that the adaptor oligo $R_oR_idT_{17}$ (5 pmol) was used as a primer and after synthesis the reaction was diluted to 200 μL with TE pH 8 and stored at 4°C. Two μL of the cDNA was used in a PCR reaction of 50 μL using 25 pmol of class A SBE specific primers PBER1 and PBERT (see below), and thirty cycles of 94° for 1 min, 60°C for 1 min and 72°C for 3 min. If Taq polymerase was used the PCR products were cloned into pT7Blue whereas if Ultma polymerase was used the PCR products were purified by chloroform extraction, ethanol precipitation and kinased in a volume of 20 μL (and then cloned into pBSSK IIP which had been cut with EcoRV and dephosphorylated). At least four classes of cDNA were isolated, which again differed in the presence or absence of *Hind* III, *Ssp* I and *Eco*R I sites. Three of these clones were sequenced fully, however one clone could not be isolated in sufficient quantity to sequence.

The sequence of one of the clones (number 19) is shown in Figure 5. The first methionine (initiation) codon starts a short open reading frame (ORF) of 7 amino acids which is out of frame with the next predicted ORF of 882 amino acids which has a molecular mass (Mr) of approximately 100 Kd. Nucleotides 6-2996 correspond to SBE sequence - the rest of the sequence shown is vector derived. Figure 6 shows a comparison of the most highly conserved part of the amino acid sequence of potato class A SBE (residues 180-871, top, row) and potato class B SBE (bottom row, residues 98-792); the middle row indicates the

degree of similarity, identical residues being denoted by the common letter, conservative changes by two dots and neutral changes by a single dot. Dashes indicate gaps introduced to optimise the alignment. The class A SBE protein has 44% identity over the entire length with potato class B SBE, and 56% identity therewith in the central conserved domain (Figure 6), as judged by the "Megalign" program (DNASTAR). However, Figure 7 shows a comparison between potato class A SBE (top row, residues 1-873) and pea class A SBE (bottom row, residues 1-861), from which it can be observed that cloned potato gene is more homologous to the class A pea enzyme, where the identity is 70 % over nearly the entire length, and this increases to 83 % over the central conserved region (starting at IPPP at position ~170). It is clear from this analysis that this cloned potato SBE gene belongs to the class A family of SBE genes.

An *E. coli* culture, containing the plasmid pSJ78 (which directs the expression of a full length potato SBE Class A gene), has been deposited (on 3rd January 1996) under the terms of the Budapest Treaty at The National Collections of Industrial and Marine Bacteria Limited (23 St Machar Drive, Aberdeen, AB2 1RY, United Kingdom), under accession number NCIMB 40781. Plasmid pSJ78 is equivalent to clone 19 described above. It represents a full length SBE A cDNA blunt-end ligated into the vector pBSSKIIP.

Polymorphism of class A SBE genes

Sequence analysis of the other two full length class A SBE genes showed that they contain frameshift mutations and are therefore unable to encode full length proteins and indeed they were unable to complement the branching enzyme deficiency in the KV832 mutant (described below). An alignment of the full length DNA sequences is shown in Figure 8: "10con.seq" (Seq ID No. 12), "19con.seq" (Seq ID No. 14) and "11con.seq" (Seq ID No. 13) represent the sequence of full length clones 10, 19 and 11 obtained by PCR using the PBER1 and PBERT primers (see below), whilst "psbe2con.seq" (Seq ID No. 18) represents the consensus sequence of the RACE clones and cDNA clone 3.2.1. Those nucleotides which differ from the overall consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. Apart from the frameshift mutations these clones are highly homologous. It should be noted that the 5' sequence of psbe2con is longer because this is the longest RACE product and it also contains several

changes compared to the other clones. The upstream methionine codon is still present in this clone but the upstream ORF is shortened to just 3 amino acids and in addition there is a 10 base deletion in the 5' untranslated leader.

The other significant area of variation is in the carboxy terminal region of the protein coding region. Closer examination of this area reveals a GAA trinucleotide repeat structure which varies in length between the four clones. These are typical characteristics of a microsatellite repeat region. The most divergent clone is #11 which has only one GAA triplet whereas clone 19 has eleven perfect repeats and the other two clones have five and seven GAA repeats. All of these deletions maintain the ORF but change the number of glutamic acid residues at the carboxy terminus of the protein.

Most of the other differences between the clones are single base changes. It is quite possible that some of these are PCR errors. To address this question direct sequencing of PCR fragments amplified from first strand cDNA was performed. Figure 9 shows the DNA sequence, and predicted amino acid sequence, obtained by such direct sequencing. Certain restriction sites are also marked. Nucleotides which could not be unambiguously assigned are indicated using standard IUPAC notation and, where this uncertainty affects the predicted amino acid sequence, a question mark is used. Sequence at the extreme 5' and 3' ends of the gene could not be determined because of the heterogeneity observed in the different cloned genes in these regions (see previous paragraph). However this can be taken as direct evidence that these differences are real and are not PCR or cloning artefacts.

There is absolutely no evidence for the frameshift mutations in the PCR derived sequence and it would appear that these mutations are an artefact of the cloning process, resulting from negative selection pressure in *E. coli*. This is supported by the fact that it proved extremely difficult to clone the full length PCR products intact as many large deletions were seen and the full length clones obtained were all cloned in one orientation (away from the LacZ promoter), perhaps suggesting that expression of the gene is toxic to the cells. Difficulties of this nature may have been responsible, at least in part, for the previous failure of other researchers to obtain the present invention.

A comparison of all the full length sequences is shown in Figure 10. In addition to clones 10, 11 and 19 are shown the sequences of a *Bgl* II - *Xho* I product cloned directly into the QE32 expression vector ("86CON.SEQ", Seq ID No. 16) and the consensus sequence of the directly sequenced PCR products ("pcrsbe2con.seq", Seq ID No. 17). Those nucleotides which differ from the consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. There are 11 nucleotide differences predicted to be present in the mRNA population, which are indicated by asterisks above and below the sequence. The other differences are probably PCR artefacts or possibly sequencing errors.

Complementation of a branching enzyme deficient *E. coli* mutant

To determine if the isolated SBE gene encodes an active protein i.e. one that has branching enzyme activity, a complementation test was performed in the *E. coli* strain KV832. This strain is unable to make bacterial glycogen as the gene for the glycogen branching enzyme has been deleted (Keil *et al.*, 1987 Mol. Gen. Genet. *207*, 294-301). When wild type cells are grown in the presence of glucose they synthesise glycogen (a highly branched glucose polymer) which stains a brown colour with iodine, whereas the KV832 cells make only a linear chain glucose polymer which stains blueish green with iodine. To determine if the cloned SBE gene could restore the ability of the KV832 cells to make a branched polymer, the clone pSJ90 (Seq ID No. 19) was used and constructed as below. The construct is a PCR-derived, substantially full length fragment (made using primers PBE 2B and PBE 2X, detailed below), which was cut with *Bgl* II and *Xho* I and cloned into the *Bam*H I / *Sal* I sites of the His-tag expression vector pQE32 (Qiagen). This clone, pSJ86, was sequenced and found to have a frameshift mutation of two bases in the 5' half of the gene. This frameshift was removed by digestion with *Nsi* I and *Sna*B I and replaced with the corresponding fragment from a Taq-generated PCR clone to produce the plasmid pSJ90 (sequence shown in Figure 12; the first 10 amino acids are derived from the expression vector). The polypeptide encoded by pSJ90 would be predicted to correspond to amino acids 46-882 of the full SBE coding sequence. The construct pSJ90 was transformed into the branching enzyme deficient KV832 cells and transformants were grown on solid PYG medium (0.85% KH_2PO_4, 1.1% K_2HPO_4, 0.6% yeast extract) containing 1.0% glucose. To test for complementation, a loop of cells was

scraped off and resuspended in 150μl of water. to which was added 15μl Lugol's solution (2g KI and 1g I_2 per 300ml water). It was found that the potato SBE fragment-transformed KV832 cells now stained a yellow-brown colour with iodine whereas control cells containing only the pQE32 vector continued to stain blue-green.

Expression of potato class A SBE in *E. coli*

Single colonies of KV832, containing one of the plasmids pQE32, pAGCR1 or pSJ90, were picked into 50ml of 2xYT medium containing carbenicillin, kanamycin and streptomycin as appropriate (100, 50 and 25 mg/L, respectively) in a 250ml flask and grown for 5 hours, with shaking, at 37°C. IPTG was then added to a final concentration of 1mM to induce expression and the flasks were further incubated overnight at 25°C. The cells were harvested by centrifugation and resuspended in 50 mM sodium phosphate buffer (pH 8.0), containing 300mM NaCl, 1mg/ml lysozyme and 1mM PMSF and left on ice for 1 hour. The cell lysates were then sonicated (3 pulses of 10 seconds at 40% power using a microprobe) and cleared by centrifugation at 12,000g for 10 minutes at 4°C. Cleared lysates were concentrated approximately 10 fold in a Centricon™ 30 filtration unit. Duplicate 10μl samples of the resulting extract were assayed for SBE activity by the phosphorylation stimulation method, as described in International Patent Application No. PCT/GB95/00634. In brief, the standard assay reaction mixture (0.2ml) was 200mM 2-(N-morpholino) ethanesulphonic acid (MES) buffer pH6.5, containing 100nCi of ^{14}C glucose-1-phosphate at 50mM, 0.05 mg rabbit phosphorylase A, and *E. coli* lysate. The reaction mixture was incubated for 60 minutes at 30°C and the reaction terminated and glucan polymer precipitated by the addition of 1ml of 75% (v/v) methanol, 1% (w/v) potassium hydroxide, and then 0.1ml glycogen (10mg/ml). The results are presented below:

Construct	SBE Activity (cpm)
pQE32 (control)	1,829
pSJ90 (potato class A SBE)	14,327
pAGCR1 (pea class A SBE)	29,707

The potato class A SBE activity is 7-8 fold above background levels. It was concluded therefore that the potato class A SBE gene was able to complement the BE mutation in the

phosphorylation stimulation assay and that the cloned gene does indeed code for a protein with branching enzyme activity.

Oligonucleotides

The following synthetic oligonucleotides (Seq ID No.s 1-11 respectively) were used:

$R_OR_IdT_{17}$	AAGGATCCGTCGACATCGATAATACGACTCACTATAGGGA$(T)_{17}$
R_O	AAGGATCCGTCGACATC
R_I	GACATCGATAATACGAC
POTSBE24	CATCCAACCACCATCTCGCA
POTSBE25	TTGAGAGAAGATACCTAAGT
POTSBE28	ATGTTCAGTCCATCTAAAGT
POTSBE29	AGAACAACAATTCCTAGCTC
PBER 1	GGGGCCTTGAACTCAGCAAT
PBERT	CGTCCCAGCATTCGACATAA
PBE 2B	CTTGGATCCTTGAACTCAGCAATTTG
PBE 2X	TAACTCGAGCAACGCGATCACAAGTTCGT

Example 2

Production of Transgenic Plants

Construction of plant transformation vectors with antisense starch branching enzyme genes

A 1200 bp *Sac* I - *Xho* I fragment, encoding approximately the -COOH half of the potato class A SBE (isolated from the rescued λZap clone 3.2.1), was cloned into the *Sac* I - *Sal* I sites of the plant transformation vector pSJ29 to create plasmid pSJ64, which is illustrated schematically in Figure 11. In the figure, the black line represents the DNA sequence. The broken line represents the bacterial plasmid backbone (containing the origin of replication and bacterial selection marker), which is not shown in full. The filled triangles on the line denote the T-DNA borders (RB = right border, LB = left border). Relevant restriction sites are shown above the black line, with the approximate distances (in kilobases) between the sites (marked by an asterisk) given by the numerals below the

line. The thinnest arrows indicate polyadenylation signals (pAnos = nopaline synthase, pAg7 = Agrobacterium gene 7), the arrows intermediate in thickness denote protein coding regions (SBE II = potato class A SBE, HYG = hygromycin resistance gene) and the thickest arrows represent promoter regions (P-2x35 = double CaMV 35S promoter, Pnos = nopaline synthase promoter). Thus pSJ64 contained the class A SBE gene fragment in an antisense orientation between the 2X 35S CaMV promoter and the nopaline synthase polyadenylation signal.

For information, pSJ29 is a derivative of the binary vector pGPTV-HYG (Becker *et al.*, 1992 Plant Molecular Biology *20*, 1195-1197) modified as follows: an approximately 750 bp (*Sac* I, T4 DNA polymerase blunted - *Sal* I) fragment of pJIT60 (Guerineau *et al.*, 1992 Plant Mol. Biol. *18*, 815-818) containing the duplicated cauliflower mosaic virus (CaMV) 35S promoter (Cabb-JI strain, equivalent to nucleotides 7040 to 7376 duplicated upstream of 7040 to 7433, Frank *et al.*, 1980 Cell *21*, 285-294) was cloned into the *Hind* III (Klenow polymerase repaired) - *Sal* I sites of pGPTV-HYG to create pSJ29.

Plant transformation

Transformation was conducted on two types of potato plant explants; either wild type untransformed minitubers (in order to give single transformants containing the class A antisense construct alone) or minitubers from three tissue culture lines (which gave rise to plants #12, #15, #17 and #18 indicated in Table 1) which had already been successfully transformed with the class B (SBE I) antisense construct containing the tandem 35S promoter (so as to obtain double transformant plants, containing antisense sequences for both the class A and class B enzymes).

Details of the method of Agrobacterium transformation, and of the growth of transformed plants, are described in International Patent Application No. WO 95/26407, except that the medium used contained 3% sucrose (not 1%) until the final transfer and that the initial incubation with Agrobacterium (strain 3850) was performed in darkness. Transformants containing the class A antisense sequence were selected by growth in medium containing 15mg/L hygromycin (the class A antisense construct comprising the HYG gene, i.e. hygromycin phosphotransferase).

Transformation was confirmed in all cases by production of a DNA fragment from the antisense gene after PCR in the presence of appropriate primers and a crude extract of genomic DNA from each regenerated shoot.

Characterisation of starch from potato plants

Starch was extracted from plants as follows: potato tubers were homogenised in water for 2 minutes in a Waring blender operating at high speed. The homogenate was washed and filtered (initially through 2mm, then through 1mm filters) using about 4 litres of water per 100gms of tubers (6 extractions). Washed starch granules were finally extracted with acetone and air dried.

Starch extracted from singly transformed potato plants (class A/SBE II antisense, or class B/SBE I antisense), or from double transformants (class A/SBE II and class B/SBE I antisense), or from untransformed control plants, was partially characterised. The results are shown in Table 1. The table shows the amount of SBE activity (units/gram tissue) in tubers from each transformed plant. The endotherm peak temperature (°C) of starch extracted from several plants was determined by DSC, and the onset temperature (°C) of pasting was determined by reference to a viscoamylograph ("RVA"), as described in WO 95/26407. The viscoamylograph profile was as follows: step 1 - 50°C for 2 minutes; step 2 - increase in temperature from 50°C to 95°C at a rate of 1.5°C per minute; step 3 - holding at 95°C for 15 minutes; step 4 - cooling from 95°C to 50°C at a rate of 1.5°C per minute; and finally, step 5 - holding at 50°C for 15 minutes. Table 1 shows the peak, pasting and set-back viscosities in stirring number units (SNUs), which is a measure of the amount of torque required to stir the suspensions. Peak viscosity may be defined for present purposes as the maximun viscosity attained during the heating phase (step 2) or the holding phase (step 3) of the viscoamylograph. Pasting viscosity may be defined as the viscosity attained by the starch suspensions at the end of the holding phase (step 3) of the viscoamylograph. Set-back viscosity may be defined as the viscosity of the starch suspension at the end of step 5 of the viscoamylograph.

A determination of apparent amylose content (% w/w) was also performed, using the iodometric assay method of Morrison & Laignelet (1983 J. Cereal Sci. *1*, 9-20). The

results (percentage apparent amylose) are shown in Table 1. The untransformed and transformed control plants gave rise to starches having apparent amylose contents in the range 29(+/-3)%.

Generally similar values for amylose content were obtained for starch extracted from most of the singly transformed plants containing the class A (SBE II) antisense sequence. However, some plants (#152, 249) gave rise to starch having an apparent amylose content of 37-38%, notably higher than the control value. Starch extracted from these plants had markedly elevated pasting onset temperatures, and starch from plant 152 also exhibited an elevated endotherm peak temperature (starch from plant 249 was not tested by DSC).

Table 1

Sample description	Sample number	Tuber SBE activity (U/g starch)	DSC Peak temperature (°C)	Viscoamylograph (RVA) Onset temperature (°C)	Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)	Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
Untransformed control	146	7.6	65.6	65.5	545	161	260	31.2	68
	243	22.2	nd	62.6	761	135	241	29.1	
AS-Class A SBE	152	12.7	69.5	70.9	467	380	529	37.5	69
	249	13.9	nd	70.0	497	434	518	38.5	
AS-Class B SBE (17) (control)	145	0.7	66.9	66.8	669	177	305	29.8	111
AS-Class B SBE (17) + AS-Class A SBE	150	0.6	74.0	66.0	214	214	303	53.1	198
	161	0.5	73.0	76.6	349	324	618	40.9	206
AS-Class B SBE (16) (control)	144	1.6	64.5	64.7	714	154	258	29.0	97
AS-Class B SBE (16) + AS-Class A SBE	149	3.0	66.5	69.9	474	267	482	35.6	127
AS-Class B SBE (15) (control)	172	0.22	nd	65.4	707	167	290	28.6	130
AS-Class B SBE (15) + AS-Class A SBE	201	0.10	nd	>95	no peak	12	13	66.4	210
	206a	0.10	nd	>95	no peak	15	17	64.1	
	206	0.30	72.6-80.5	>95	no peak	14	19	62.8	240
	202	0.02	nd	69.4	no peak	172	245	57.9	
	212	1.40	nd	78.0	306	296	541	49.5	
	220	1.40	nd	75.6	355	345	593	44.1	
AS-Class B SBE (12) (control)	170	0.2	nd	66.5	768	202	303	27.6	
AS-Class B SBE (12) + AS-Class A SBE	238	0.7	nd	95.0	no peak	23	14	60.4	
	238a	0.9	nd	91.2	no peak	139	192	56.7	
	230a	0.6	nd	77.6	244	239	450	48.2	

RVA profile	50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (15 min)
Pasting viscosity (47 min)	at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)
Set-back viscosity (92 min)	at end of profile
SBE	Starch Branching Enzyme
SNU	Instrument "Stirring Number Units" (arbitrary units)
nd	not determined

Table 1

Sample description	Sample number	Tuber SBE activity (U/g starch)	DSC Peak temperature (°C)	DSC Onset temperature (°C)
Untransformed control	146	7.6	65.8	65.5
	243	22.2	nd	62.6
AS-Class A SBE	152	12.7	69.5	70.9
	249	13.9	nd	70.0
AS-Class B SBE (17) (control)	145	0.7	66.9	66.8
AS-Class B SBE (17) + AS-Class A SBE	150	0.6	74.0	86.0
	161	0.5	73.0	76.6
AS-Class B SBE (18) (control)	144	1.6	64.5	64.7
AS-Class B SBE (18) + AS-Class A SBE	149	3.0	68.5	69.9

Viscoamylograph (RVA)			Apparent amylose content (% w/w)	Phosphorus content (mg/100g)
Peak viscosity (SNU)	Pasting viscosity (SNU)	Set-back viscosity (SNU)		
545	161	280	31.2	68
761	135	241	29.1	
467	390	529	37.5	89
497	434	518	38.5	
669	177	305	29.8	111
214	214	303	53.1	198
349	324	618	40.9	206
714	154	258	29.0	97
474	267	482	35.6	127

AS-Class B SBE (15) (control)	172	0.22	nd	65.4
AS-Class B SBE (15) + AS-Class A SBE	201	0.10	nd	>95
	208a	0.10	nd	>95
	208	0.30	72.8-80.5	>95
	202	0.02	nd	89.4
	212	1.40	nd	78.0
	220	1.40	nd	75.8
AS-Class B SBE (12) (control)	170	0.2	nd	66.5
AS-Class B SBE (12) + AS-Class A SBE	236	0.7	nd	95.0
	236a	0.9	nd	91.2
	230a	0.8	nd	77.6

RVA profile — 50°C (2 min), 50-95°C (1.5°C/min), 95°C (15 min), 95-50°C (1.5°C/min), 50°C (15 min)

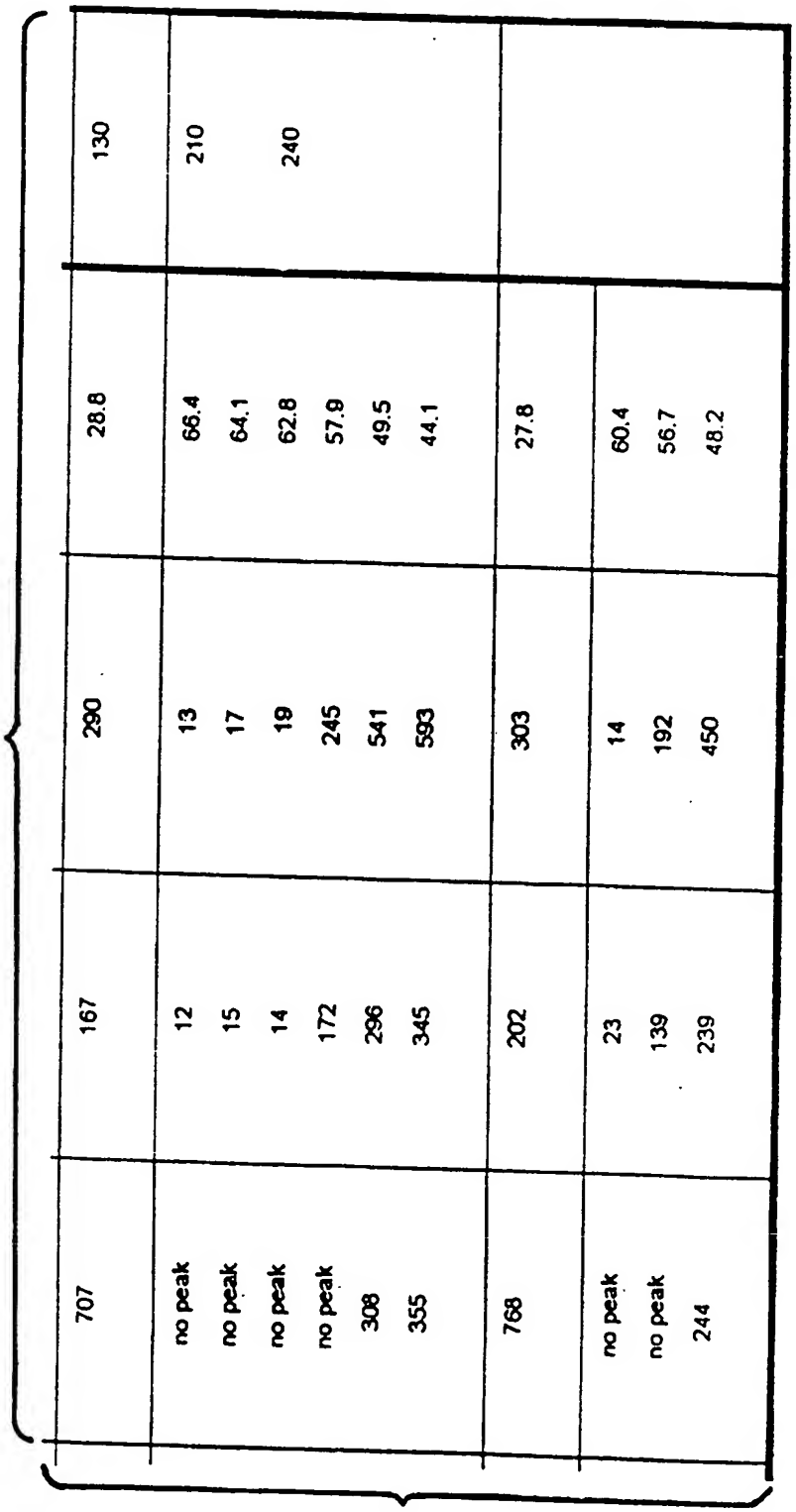
Pasting viscosity (47 min) — at end of 50°C (2min), 50-95°C (1.5°C/min), 95°C (15 min)

Set-back viscosity (92 min) — at end of profile

SBE — Starch Branching Enzyme

SNU — Instrument "Stirring Number Units" (arbitrary units)

nd — not determined



707	167	290	28.8	130
no peak	12	13	66.4	210
no peak	15	17	64.1	
no peak	14	19	62.8	240
no peak	172	245	57.9	
308	296	541	49.5	
355	345	593	44.1	
768	202	303	27.8	
no peak	23	14	60.4	
no peak	139	192	56.7	
244	239	450	48.2	

It should be noted that, even if other single transformants were not to provide starch with an altered amylose/amylopectin ratio, the starch from such plants might still have different properties relative to starch from conventional plants (e.g. different average molecular weight or different amylopectin branching patterns), which might be useful.

Double transformant plants, containing antisense sequences for both the class A and class B enzymes, had greatly reduced SBE activity (units/gm) compared to untransformed plants or single anti-sense class A transformants, (as shown in Table 1). Moreover, certain of the double transformant plants contained starch having very significantly altered properties. For example, starch extracted from plants #201, 202, 208, 208a, 236 and 236a had drastically altered amylose/amylopectin ratios, to the extent that amylose was the main constituent of starch from these plants. The pasting onset temperatures of starch from these plants were also the most greatly increased (by about 25-30°C). Starch from plants such as #150, 161, 212, 220 and 230a represented a range of intermediates, in that such starch displayed a more modest rise in both amylose content and pasting onset temperature. The results would tend to suggest that there is generally a correlation between % amylose content and pasting onset temperature, which is in agreement with the known behaviour of starches from other sources, notably maize.

The marked increase in amylose content obtained by inhibition of class A SBE alone, compared to inhibition of class B SBE alone (see PCT/GB95/00634) might suggest that it would be advantageous to transform plants first with a construct to suppress class A SBE expression (probably, in practice, an antisense construct), select those plants giving rise to starch with the most altered properties, and then to re-transform with a construct to suppress class B SBE expression (again, in practice, probably an antisense construct), so as to maximise the degree of starch modification.

In addition to pasting onset temperatures, other features of the viscoamylograph profile e.g. for starches from plants #149, 150, 152, 161, 201, 236 and 236a showed significant differences to starches from control plants, as illustrated in Figure 13. Referring to Figure 13, a number of viscoamylograph traces are shown. The legend is as follows: shaded box - normal potato starch control (29.8% amylose content); shaded circle - starch from plant

149 (35.6% amylose): shaded triangle, pointing upwards - plant 152 (37.5%); shaded triangle, pointing downwards - plant 161 (40.9%); shaded diamond - plant 150 (53.1%); unshaded box - plant 236a (56.7%); unshaded circle - plant 236 (60.4%); unshaded triangle, pointing upwards - plant 201 (66.4%); unshaded triangle, pointing downwards - Hylon V starch, from maize (44.9 % amylose). The thin line denotes the heating profile.

With increasing amylose content, peak viscosities during processing to 95°C decrease, and the drop in viscosity from the peak until the end of the holding period at 95°C also generally decreases (indeed, for some of the starch samples there is an increase in viscosity during this period). Both of these results are indicative of reduced granule fragmentation, and hence *increased* granule stability during pasting. This property has not previously been available in potato starch without extensive prior chemical or physical modification. For applications where a maximal viscosity after processing to 95°C is desirable (i.e. corresponding to the viscosity after 47 minutes in the viscoamylograph test), starch from plant #152 would be selected as starches with both lower (Controls, #149) and higher (#161, #150) amylose contents have lower viscosities following this gelatinisation and pasting regime (Figure 13 and Table 1). It is believed that the viscosity at this stage is determined by a combination of the extent of granule swelling and the resistance of swollen granules to mechanical fragmentation. For any desired viscosity behaviour, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing suitable standard viscosity tests.

Upon cooling pastes from 95°C to 50°C, potato starches from most plants transformed in accordance with the invention showed an increase in viscoamylograph viscosity as expected for partial reassociation of amylose. Starches from plants #149, 152 and 161 all show viscosities at 50°C significantly in excess of those for starches from control plants (Figure 13 and Table 1). This contrasts with the effect of elevated amylose contents in starches from maize plants (Figure 2) which show very low viscosities throughout the viscoamylograph test. Of particular note is the fact that, for similar amylose contents, starch from potato plant 150 (53% amylose) shows markedly increased viscosity compared with Hylon 5 starch (44.9% amylose) as illustrated in Figure 13. This demonstrates that

useful properties which require elevated (35% or greater) amylose levels can be obtained by processing starches from potato plants below 100°C, whereas more energy-intensive processing is required in order to generate similarly useful properties from high amylose starches derived from maize plants.

Final viscosity in the viscoamylograph test (set-back viscosity after 92 minutes) is greatest for starch from plant #161 (40.9% amylose) amongst those tested (Figure 13 and Table 1). Decreasing final viscosities are obtained for starches from plant #152 (37.5% amylose), #149 (35.6% amylose) and #150 (53.1% amylose). Set-back viscosity occurs where amylose molecules, exuded from the starch granule during pasting, start to re-associate outside the granule and form a viscous gel-like substance. It is believed that the set-back viscosity values of starches from transgenic potato plants represent a balance between the inherent amylose content of the starches and the ability of the amylose fraction to be exuded from the granule during pasting and therefore be available for the reassociation process which results in viscosity increase. For starches with low amylose content, increasing the amylose content tends to make more amylose available for re-association, thus increasing the set-back viscosity. However, above a threshold value, increased amylose content is thought to inhibit granule swelling, thus preventing exudation of amylose from the starch granule and reducing the amount of amylose available for re-association. This is supported by the RVA results obtained for the very high amylose content potato starches seen in the viscoamylograph profiles in Figure 13. For any desired viscosity behaviour following set-back or retrogradation to any desired temperature over any desired timescale, one skilled in the art would select a potato starch from a range containing different amylose contents produced according to the invention by performing standard viscosity tests.

Further experiments with starch from plants #201 and 208 showed that this had an apparent amylose content of over 62% (see Table 1). Viscoamylograph studies showed that starch from these plants had radically altered properties and behaved in a manner similar to hylon 5 starch from maize plants (Figure 13). Under the conditions employed in the viscoamylograph, this starch exhibited extremely limited (nearly undetectable) granule swelling. Thus, for example, unlike starch from control plants, starch from plants

201, 208 and 208a did not display a clearly defined pasting viscosity peak during the heating phase. Microscopic analysis confirmed that the starch granule structure underwent only minor swelling during the experimental heating process. This property may well be particularly useful in certain applications, as will be apparent to those skilled in the art.

Some re-grown plants have so far been found to increase still further the apparent amylose content of starch extracted therefrom. Such increases may be due to:-

i) Growth and development of the first generation transformed plants may have been affected to some degree by the exogenous growth hormones present in the tissue culture system, which exogenoous hormones were not present during growth of the second generation plants; and

ii) Subsequent generations were grown under field conditions, which may allow for attainment of greater maturity than growth under laboratory conditions, it being generally held that amylose content of potato starch increases with maturity of the potato tuber.

Accordingly, it should be possible to obtain potato plants giving rise to tubers with starch having an amylose content in excess of the 66% level so far attained, simply by analysing a greater number of transformed plants and/or by re-growing transgenic plants through one or more generations under field conditions.

Table 1 shows that another characteristic of starch which is affected by the presence of anti-sense sequences to SBE is the phosphorus content. Starch from untransformed control plants had a phosphorus content of about 60-70mg/100gram dry weight (as determined according to the AOAC Official Methods of Analysis, 15th Edition, Method 948.09 "Phosphorus in Flour"). Introduction into the plant of an anti-sense SBE B sequence was found to cause a modest increase (about two-fold) in phosphorus content, which is in agreement with the previous findings reported at scientific meetings. Similarly, anti-sense to SBE A alone causes only a small rise in phosphorus content relative to untransformed controls. However, use of anti-sense to both SBE A and B in combination results in up to a four-fold increase in phosphorus content. which is far greater than any *in planta* phosphorus content previously demonstrated for potato starch.

This is useful in that. for certain applications. starch must be phosphorylated *in vitro* by

chemical modification. The ability to obtain potato starch which, as extracted from the plant, already has a high phosphorus content will reduce the amount of *in vitro* phosphorylation required suitably to modify the starch. Thus, in another aspect the invention provides potato starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100gram dry weight starch. Typically the starch will have a phosphorus content in the range 200 - 240mg/100gram dry weight starch.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: National Starch and Chemical Investment Holding Corporation
(B) STREET: 501 Silverside Road. Suite 27
(C) CITY: Wilmington
(D) STATE: Delaware
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 19809

(ii) TITLE OF INVENTION: Improvements in or Relating to Plant Starch Composition

(iii) NUMBER OF SEQUENCES: 20

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0. Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 57 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGGATCCGT CGACATCGAT AATACGACTC ACTATAGGGA TTTTTTTTTT TTTTTTT 57

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AAGGATCCGT CGACATC 17

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACATCGATA ATACGAC 17

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CATCCAACCA CCATCTCGCA 20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TTGAGAGAAG ATACCTAAGT 20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ATGTTCAGTC CATCTAAAGT 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AGAACAACAA TTCCTAGCTC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGGGCCTTGA ACTCAGCAAT 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CGTCCCAGCA TTCGACATAA 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

CTTGGATCCT TGAACTCAGC AATTTG 26

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TAACTCGAGC AACGCGATCA CAAGTTCGT 29

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3003 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GATGGGGCCT TGAACTCAGC AATTTGACAC TCAGTTAGTT ACACTGCCAT CACTTATCAG 60

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CCCTAAGGAG AGAAGAAGAA AGATGGTGTA TACACTCTCT GGAGTTCGTT TTCCTACTGT 180

TCCATCAGTG TACAAATCTA ATGGATTCAG CAGTAATGGT GATCGGAGGA ATGCTAATAT 240

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CAATTCCGAA TCCCGACCTT CTACAATTGC AGCATCGGGG AAAGTCCTTG TGCCTGGAAT 360

CCAGAGTGAT AGCTCCTCAT CCTCAACAGA TCAATTTGAG TTCGCTGAGA CATCTCCAGA 420

AAATTCCCCA GCATCAACTG ATGTAGATAG TTCAACAATG GAACACGCTA GCCAGATTAA 480

AACTGAGAAC GATGACGTTG AGCCGTCAAG TGATCTTACA GGAAGTGTTG AAGAGCTGGA 540

TTTTGCTTCA TCACTACAAC TACAAGAAGG TGGTAAACTG GAGGAGTCTA AAACATTAAA 600

TACTTCTGAA GAGACAATTA TTGATGAATC TGATAGGATC AGAGAGAGGG GCATCCCTCC 660

ACCTGGACTT GGTCAGAAGA TTTATGAAAT AGACCCCCTT TTGACAAACT ATCGTCAACA 720

CCTTGATTAC AGGTATTCAC AGTACAAGAA ACTGAGGGAG GCAATTGACA AGTATGAGGG 780

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TCCCAGATGC AATTACCATT GGTGAAGATG TTAGCGGAAT GCCGACATTT TGTGTTCCCG 1860

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CAAAAACATA TGTAAAATCG ATGAATTTAT GTCGAATGCT GGGACGATCG AATTCCTGCA 3000

GCC 3003

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2975 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TTGATGGGCC TTGAACTCAG CAATTTGACA CTCAGTTAGT TACACTCCTA TCACTTATCA 60

GATCTCTATT TTTTCTCTTA ATTCCAACCA GGGGAATGAA TAAAAGGATA GATTTGTAAA 120

AACCCTAAGG AGAGAAGAAG AAAGATGGTG TATATACTCT CTGGAGTTCG TTTTCCTACT 180

GTTCCATCAG TGTACAAATC TAATGGATTC AGCAGTAATG GTGATCGGAG GAATGCTAAT 240

GTTTCTGTAT TCTTGAAAAA GCACTCTCTT TCACGGAAGA TCTTGGCTGA AAAGTCTTCT 300

TACAATTCCG AATTCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT TGTGCCTGGA 360

ACCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTTG AGTTCACTGA GACATCTCCA 420

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AAAACTGAGA ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT TGAAGAGCTG 540

GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC TAAAACATTA 600

AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG GGGCATCCCT 660

CCACCTGGAC TTGGTCAGAA GATTTATGAA ATAGACCCCC TTTTGACAAA CTATCGTCAA 720

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ACGGCACCGA TAGTTGTTAC TTTCACTCTG GAGCTCGTGG TTATCATTGG ATGTGGGATT 1560

CCGCCTCTTT AACTATGGAA ACTGGGAGGT ACTTAGGTAT CTTCTCTCAA ATGCGAGATG 1620

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AACTGATGTG GATGCTGTTG TGTATCTGAT GCTGGTCAAC GATCTTATTC ATAGGCTTTT 1800

CCCAGATGCA ATTACCATTG GTGAAGATGT TAGCGGAATG CCGACATTTT GTATTCCCGT 1860

TCAAGATGGG GGTGTTGGCT TTGACTATCG GCTGCATATG GCAATTGCTG ATAAATGGAT 1920

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AAATAGAAGA TGGTCGGAAA AGTGTGTTTC ATACGCTGAA AGTCATGATC AAGCTCTAGT 2040

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GGATAGACCG CCAACATCAT TAATAGATCG TGGGATAGCA TTGCACAAGA TGATTAGGCT 2160

TGTAACTATG GGATTAGGAG GAGAAGGGTA CCTAAATTTC ATGGGAAATG AATTCGGCCA 2220

CCCTGAGTGG ATTGATTTCC CTAGGGCTGA GCCACACCTT TCTGATGGCT CAGTAATTCC 2280

CGGAAACCAA TTCAGTTATG ATAAATGCAG ACGGAGATTT GACCTGGGAG ATGCAGAATA 2340

TTTAAGATAC CATGGGTTAC AAGAATTTGA CTGGGCTATG CAGTATCTTG AAGATAAATA 2400

TGAGTTTATG ACTTCAGAAC ACCAGTTCAT ATCACGAAAG GATGAAGGAG ATAGGATGAT 2460

TGTATTTGAA AGAGGAAACC TAGTTTTCGT CTTTAATTTT CACTGGACAA ATAGCTATTC 2520

AGACTATCGC ATAGGCTGCC TGAAGCCTGG AAAATACAAG GTTGTCTTGG ACTCAGATGA 2580

TCCACTTTTT GGTGGCTTCG GGAGAATTGA TCATAATGCC GAATATTTCA CCTCTGAAGG 2640

ATCGTATGAT GATCGTCCTT GTTCAATTAT GGTGTATGCA CCTAGTAGAA CAGCAGTGGT 2700

CTATGCACTA GTAGACAAAC TAGAAGTAGC AGTAGTAGAA GAACCCATTG AAGAATGAAC 2760

GAACTTGTGA TCGCGTTGAA AGATTTGAAC GTTACTTGGT CATCCACATA GAGCTTCTTG 2820

ACATCAGTCT TGGCGGAATT GCATGTGACA ACAAGGTTTG CAGTTCTTTC CACTATTAGT 2880

AGTCCACCGA TATACGCAGA GATGAAGTGC TGAACAAACA TATGTAAAAT CGATGAATTT 2940

ATGTCGAATG CTGGGACGAT CGAATTCCTG CAGCC 2975

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3033 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:145..2790

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

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TTGATGGGGC CTTGAACTCA GCAATTTGAC ACTCAGTTAG TTACACTCCT ATCACTTATC      60

AGATCTCTAT TTTTTCTCTT AATTCCAACC AAGGAATGAA TAAAAGGATA GATTTGTAAA     120

AACCCTAAGG AGAGAAGAAG AAAG ATG GTG TAT ACA CTC TCT GGA GTT CGT         171
                           Met Val Tyr Thr Leu Ser Gly Val Arg
                             1               5

TTT CCT ACT GTT CCA TCA GTG TAC AAA TCT AAT GGA TTC AGC AGT AAT       219
Phe Pro Thr Val Pro Ser Val Tyr Lys Ser Asn Gly Phe Ser Ser Asn
 10                  15                  20                  25

GGT GAT CGG AGG AAT GCT AAT GTT TCT GTA TTC TTG AAA AAG CAC TCT       267
Gly Asp Arg Arg Asn Ala Asn Val Ser Val Phe Leu Lys Lys His Ser
                 30                  35                  40

CTT TCA CGG AAG ATC TTG GCT GAA AAG TCT TCT TAC AAT TCC GAA TTC       315
Leu Ser Arg Lys Ile Leu Ala Glu Lys Ser Ser Tyr Asn Ser Glu Phe
             45                  50                  55

CGA CCT TCT ACA GTT GCA GCA TCG GGG AAA GTC CTT GTG CCT GGA ACC       363
Arg Pro Ser Thr Val Ala Ala Ser Gly Lys Val Leu Val Pro Gly Thr
         60                  65                  70

CAG AGT GAT AGC TCC TCA TCC TCA ACA GAC CAA TTT GAG TTC ACT GAG       411
Gln Ser Asp Ser Ser Ser Ser Ser Thr Asp Gln Phe Glu Phe Thr Glu
     75                  80                  85

ACA TCT CCA GAA AAT TCC CCA GCA TCA ACT GAT GTA GAT AGT TCA ACA       459
Thr Ser Pro Glu Asn Ser Pro Ala Ser Thr Asp Val Asp Ser Ser Thr
 90                  95                 100                 105

ATG GAA CAC GCT AGC CAG ATT AAA ACT GAG AAC GAT GAC GTT GAG CCG       507
Met Glu His Ala Ser Gln Ile Lys Thr Glu Asn Asp Asp Val Glu Pro
                110                 115                 120

TCA AGT GAT CTT ACA GGA AGT GTT GAA GAG CTG GAT TTT GCT TCA TCA       555
Ser Ser Asp Leu Thr Gly Ser Val Glu Glu Leu Asp Phe Ala Ser Ser
            125                 130                 135
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CTA CAA CTA CAA GAA GGT GGT AAA CTG GAG GAG TCT AAA ACA TTA AAT      603
Leu Gln Leu Gln Glu Gly Gly Lys Leu Glu Glu Ser Lys Thr Leu Asn
        140                 145                 150

ACT TCT GAA GAG ACA ATT ATT GAT GAA TCT GAT AGG ATC AGA GAG AGG      651
Thr Ser Glu Glu Thr Ile Ile Asp Glu Ser Asp Arg Ile Arg Glu Arg
    155                 160                 165

GGC ATC CCT CCA CCT GGA CTT GGT CAG AAG ATT TAT GAA ATA GAC CCC      699
Gly Ile Pro Pro Pro Gly Leu Gly Gln Lys Ile Tyr Glu Ile Asp Pro
170                 175                 180                 185

CTT TTG ACA AAC TAT CGT CAA CAC CTT GAT TAC AGG TAT TCA CAG TAC      747
Leu Leu Thr Asn Tyr Arg Gln His Leu Asp Tyr Arg Tyr Ser Gln Tyr
                190                 195                 200

AAG AAA CTG AGG GAG GCA ATT GAC AAG TAT GAG GGT GGT TTG GAA GCC      795
Lys Lys Leu Arg Glu Ala Ile Asp Lys Tyr Glu Gly Gly Leu Glu Ala
            205                 210                 215

TTT TCT CGT GGT TAT GAA AAA ATG GGT TTC ACT CGT AGT GCT ACA GGT      843
Phe Ser Arg Gly Tyr Glu Lys Met Gly Phe Thr Arg Ser Ala Thr Gly
        220                 225                 230

ATC ACT TAC CGT GAG TGG GCT CTT GGT GCC CAG TCA GCT GCC CTC ATT      891
Ile Thr Tyr Arg Glu Trp Ala Leu Gly Ala Gln Ser Ala Ala Leu Ile
    235                 240                 245

GGA GAT TTC AAC AAT TGG GAC GCA AAT GCT GAC ATT ATG ACT CGG AAT      939
Gly Asp Phe Asn Asn Trp Asp Ala Asn Ala Asp Ile Met Thr Arg Asn
250                 255                 260                 265

GAA TTT GGT GTC TGG GAG ATT TTT CTG CCA AAT AAT GTG GAT GGT TCT      987
Glu Phe Gly Val Trp Glu Ile Phe Leu Pro Asn Asn Val Asp Gly Ser
                270                 275                 280

CCT GCA ATT CCT CAT GGG TCC AGA GTG AAG ATA CGT ATG GAC ACT CCA     1035
Pro Ala Ile Pro His Gly Ser Arg Val Lys Ile Arg Met Asp Thr Pro
            285                 290                 295

TCA GGT GTT AAG GAT TCC ATT CCT GCT TGG ATC AAC TAC TCT TTA CAG     1083
Ser Gly Val Lys Asp Ser Ile Pro Ala Trp Ile Asn Tyr Ser Leu Gln
        300                 305                 310

CTT CCT GAT GAA ATT CCA TAT AAT GGA ATA CAT TAT GAT CCA CCC GAA     1131
Leu Pro Asp Glu Ile Pro Tyr Asn Gly Ile His Tyr Asp Pro Pro Glu
    315                 320                 325

GAG GAG AGG TAT ATC TTC CAA CAC CCA CGG CCA AAG AAA CCA AAG TCG     1179
Glu Glu Arg Tyr Ile Phe Gln His Pro Arg Pro Lys Lys Pro Lys Ser
330                 335                 340                 345

CTG AGA ATA TAT GAA TCT CAT ATT GGA ATG AGT AGT CCG GAG CCT AAA     1227
Leu Arg Ile Tyr Glu Ser His Ile Gly Met Ser Ser Pro Glu Pro Lys
                350                 355                 360
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ATT AAC TCA TAC GTG AAT TTT AGA GAT GAA GTT CTT CCT CGC ATA AAA      1275
Ile Asn Ser Tyr Val Asn Phe Arg Asp Glu Val Leu Pro Arg Ile Lys
           365                 370                 375

AAG CTT GGG TAC AAT GCG CTG CAA ATT ATG GCT ATT CAA GAG CAT TCT      1323
Lys Leu Gly Tyr Asn Ala Leu Gln Ile Met Ala Ile Gln Glu His Ser
       380                 385                 390

TAT TAC GCT AGT TTT GGT TAT CAT GTC ACA AAT TTT TTT GCA CCA AGC      1371
Tyr Tyr Ala Ser Phe Gly Tyr His Val Thr Asn Phe Phe Ala Pro Ser
   395                 400                 405

AGC CGT TTT GGA ACG CCC GAC GAC CTT AAG TCT TTG ATT GAT AAA GCT      1419
Ser Arg Phe Gly Thr Pro Asp Asp Leu Lys Ser Leu Ile Asp Lys Ala
410                 415                 420                 425

CAT GAG CTA GGA ATT GTT GTT CTC ATG GAC ATT GTT CAC AGC CAT GCA      1467
His Glu Leu Gly Ile Val Val Leu Met Asp Ile Val His Ser His Ala
               430                 435                 440

TCA AAT AAT ACT TTA GAT GGA CTG AAC ATG TTT GAC TGC ACC GAT AGT      1515
Ser Asn Asn Thr Leu Asp Gly Leu Asn Met Phe Asp Cys Thr Asp Ser
           445                 450                 455

TGT TAC TTT CAC TCT GGA GCT CGT GGT TAT CAT TGG ATG TGG GAT TCC      1563
Cys Tyr Phe His Ser Gly Ala Arg Gly Tyr His Trp Met Trp Asp Ser
       460                 465                 470

CGC CTC TTT AAC TAT GGA AAC TGG GAG GTA CTT AGG TAT CTT CTC TCA      1611
Arg Leu Phe Asn Tyr Gly Asn Trp Glu Val Leu Arg Tyr Leu Leu Ser
   475                 480                 485

AAT GCG AGA TGG TGG TTG GAT GCG TTC AAA TTT GAT GGA TTT AGA TTT      1659
Asn Ala Arg Trp Trp Leu Asp Ala Phe Lys Phe Asp Gly Phe Arg Phe
490                 495                 500                 505

GAT GGT GTG ACA TCA ATG ATG TAT ATT CAC CAC GGA TTA TCG GTG GGA      1707
Asp Gly Val Thr Ser Met Met Tyr Ile His His Gly Leu Ser Val Gly
               510                 515                 520

TTC ACT GGG AAC TAC GAG GAA TAC TTT GGA CTC GCA ACT GAT GTG GAT      1755
Phe Thr Gly Asn Tyr Glu Glu Tyr Phe Gly Leu Ala Thr Asp Val Asp
           525                 530                 535

GCT GTT GTG TAT CTG ATG CTG GTC AAC GAT CTT ATT CAT GGG CTT TTC      1803
Ala Val Val Tyr Leu Met Leu Val Asn Asp Leu Ile His Gly Leu Phe
       540                 545                 550

CCA GAT GCA ATT ACC ATT GGT GAA GAT GTT AGC GGA ATG CCG ACA TTT      1851
Pro Asp Ala Ile Thr Ile Gly Glu Asp Val Ser Gly Met Pro Thr Phe
   555                 560                 565

TGT ATT CCC GTC CAA GAG GGG GGT GTT GGC TTT GAC TAT CGG CTG CAT      1899
Cys Ile Pro Val Gln Glu Gly Gly Val Gly Phe Asp Tyr Arg Leu His
570                 575                 580                 585
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ATG GCA ATT GCT GAT AAA CGG ATT GAG TTG CTC AAG AAA CGG GAT GAG      1947
Met Ala Ile Ala Asp Lys Arg Ile Glu Leu Leu Lys Lys Arg Asp Glu
                590                 595                 600

GAT TGG AGA GTG GGT GAT ATT GTT CAT ACA CTG ACA AAT AGA AGA TGG      1995
Asp Trp Arg Val Gly Asp Ile Val His Thr Leu Thr Asn Arg Arg Trp
            605                 610                 615

TCG GAA AAG TGT GTT TCA TAC GCT GAA AGT CAT GAT CAA GCT CTA GTC      2043
Ser Glu Lys Cys Val Ser Tyr Ala Glu Ser His Asp Gln Ala Leu Val
        620                 625                 630

GGT GAT AAA ACT ATA GCA TTC TGG CTG ATG GAC AAG GAT ATG TAT GAT      2091
Gly Asp Lys Thr Ile Ala Phe Trp Leu Met Asp Lys Asp Met Tyr Asp
    635                 640                 645

TTT ATG GCT CTG GAT AGA CCG TCA ACA TCA TTA ATA GAT CGT GGG ATA      2139
Phe Met Ala Leu Asp Arg Pro Ser Thr Ser Leu Ile Asp Arg Gly Ile
650                 655                 660                 665

GCA TTG CAC AAG ATG ATT AGG CTT GTA ACT ATG GGA TTA GGA GGA GAA      2187
Ala Leu His Lys Met Ile Arg Leu Val Thr Met Gly Leu Gly Gly Glu
                670                 675                 680

GGG TAC CTA AAT TTC ATG GGA AAT GAA TTC GGC CAC CCT GAG TGG ATT      2235
Gly Tyr Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Glu Trp Ile
            685                 690                 695

GAT TTC CCT AGG GCT GAA CAA CAC CTC TCT GAT GGC TCA GTA ATC CCC      2283
Asp Phe Pro Arg Ala Glu Gln His Leu Ser Asp Gly Ser Val Ile Pro
        700                 705                 710

GGA AAC CAA TTC AGT TAT GAT AAA TGC AGA CGG AGA TTT GAC CTG GGA      2331
Gly Asn Gln Phe Ser Tyr Asp Lys Cys Arg Arg Arg Phe Asp Leu Gly
    715                 720                 725

GAT GCA GAA TAT TTA AGA TAC CGT GGG TTG CAA GAA TTT GAC CGG CCT      2379
Asp Ala Glu Tyr Leu Arg Tyr Arg Gly Leu Gln Glu Phe Asp Arg Pro
730                 735                 740                 745

ATG CAG TAT CTT GAA GAT AAA TAT GAG TTT ATG ACT TCA GAA CAC CAG      2427
Met Gln Tyr Leu Glu Asp Lys Tyr Glu Phe Met Thr Ser Glu His Gln
                750                 755                 760

TTC ATA TCA CGA AAG GAT GAA GGA GAT AGG ATG ATT GTA TTT GAA AAA      2475
Phe Ile Ser Arg Lys Asp Glu Gly Asp Arg Met Ile Val Phe Glu Lys
            765                 770                 775

GGA AAC CTA GTT TTT GTC TTT AAT TTT CAC TGG ACA AAA AGC TAT TCA      2523
Gly Asn Leu Val Phe Val Phe Asn Phe His Trp Thr Lys Ser Tyr Ser
        780                 785                 790

GAC TAT CGC ATA GCC TGC CTG AAG CCT GGA AAA TAC AAG GTT GCC TTG      2571
Asp Tyr Arg Ile Ala Cys Leu Lys Pro Gly Lys Tyr Lys Val Ala Leu
    795                 800                 805
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GAC TCA GAT GAT CCA CTT TTT GGT GGC TTC GGG AGA ATT GAT CAT AAT      2619
Asp Ser Asp Asp Pro Leu Phe Gly Gly Phe Gly Arg Ile Asp His Asn
810                 815                 820                 825

GCC GAA TAT TTC ACC TTT GAA GGA TGG TAT GAT GAT CGT CCT CGT TCA      2667
Ala Glu Tyr Phe Thr Phe Glu Gly Trp Tyr Asp Asp Arg Pro Arg Ser
                830                 835                 840

ATT ATG GTG TAT GCA CCT TGT AAA ACA GCA GTG GTC TAT GCA CTA GTA      2715
Ile Met Val Tyr Ala Pro Cys Lys Thr Ala Val Val Tyr Ala Leu Val
            845                 850                 855

GAC AAA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA GAA GTA GCA GCA      2763
Asp Lys Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Val Ala Ala
        860                 865                 870

GTA GAA GAA GTA GTA GTA GAA GAA GAA TGAACGAACT TGTGATCGCG            2810
Val Glu Glu Val Val Val Glu Glu Glu
    875                 880

TTGAAAGATT TGAACGCTAC ATAGAGCTTC TTGACGTATC TGGCAATATT GCATCAGTCT    2870

TGGCGGAATT TCATGTGACA CAAGGTTTGC AATTCTTTCC ACTATTAGTA GTGCAACGAT    2930

ATACGCAGAG ATGAAGTGCT GAACAAACAT ATGTAAAATC GATGAATTTA TGTCGAATGC    2990

TGGGACGATC GAATTCCTGC AGGCCGGGGG ACCCCTTAGT TCT                      3033
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(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 882 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

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Met Val Tyr Thr Leu Ser Gly Val Arg Phe Pro Thr Val Pro Ser Val
 1               5                  10                  15

Tyr Lys Ser Asn Gly Phe Ser Ser Asn Gly Asp Arg Arg Asn Ala Asn
            20                  25                  30

Val Ser Val Phe Leu Lys Lys His Ser Leu Ser Arg Lys Ile Leu Ala
        35                  40                  45

Glu Lys Ser Ser Tyr Asn Ser Glu Phe Arg Pro Ser Thr Val Ala Ala
    50                  55                  60

Ser Gly Lys Val Leu Val Pro Gly Thr Gln Ser Asp Ser Ser Ser Ser
65                  70                  75                  80

Ser Thr Asp Gln Phe Glu Phe Thr Glu Thr Ser Pro Glu Asn Ser Pro
                85                  90                  95
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Ala Ser Thr Asp Val Asp Ser Ser Thr Met Glu His Ala Ser Gln Ile
100 105 110

Lys Thr Glu Asn Asp Asp Val Glu Pro Ser Ser Asp Leu Thr Gly Ser
115 120 125

Val Glu Glu Leu Asp Phe Ala Ser Ser Leu Gln Leu Gln Glu Gly Gly
130 135 140

Lys Leu Glu Glu Ser Lys Thr Leu Asn Thr Ser Glu Glu Thr Ile Ile
145 150 155 160

Asp Glu Ser Asp Arg Ile Arg Glu Arg Gly Ile Pro Pro Pro Gly Leu
165 170 175

Gly Gln Lys Ile Tyr Glu Ile Asp Pro Leu Leu Thr Asn Tyr Arg Gln
180 185 190

His Leu Asp Tyr Arg Tyr Ser Gln Tyr Lys Lys Leu Arg Glu Ala Ile
195 200 205

Asp Lys Tyr Glu Gly Gly Leu Glu Ala Phe Ser Arg Gly Tyr Glu Lys
210 215 220

Met Gly Phe Thr Arg Ser Ala Thr Gly Ile Thr Tyr Arg Glu Trp Ala
225 230 235 240

Leu Gly Ala Gln Ser Ala Ala Leu Ile Gly Asp Phe Asn Asn Trp Asp
245 250 255

Ala Asn Ala Asp Ile Met Thr Arg Asn Glu Phe Gly Val Trp Glu Ile
260 265 270

Phe Leu Pro Asn Asn Val Asp Gly Ser Pro Ala Ile Pro His Gly Ser
275 280 285

Arg Val Lys Ile Arg Met Asp Thr Pro Ser Gly Val Lys Asp Ser Ile
290 295 300

Pro Ala Trp Ile Asn Tyr Ser Leu Gln Leu Pro Asp Glu Ile Pro Tyr
305 310 315 320

Asn Gly Ile His Tyr Asp Pro Pro Glu Glu Glu Arg Tyr Ile Phe Gln
325 330 335

His Pro Arg Pro Lys Lys Pro Lys Ser Leu Arg Ile Tyr Glu Ser His
340 345 350

Ile Gly Met Ser Ser Pro Glu Pro Lys Ile Asn Ser Tyr Val Asn Phe
355 360 365

Arg Asp Glu Val Leu Pro Arg Ile Lys Lys Leu Gly Tyr Asn Ala Leu
370 375 380

Gln Ile Met Ala Ile Gln Glu His Ser Tyr Tyr Ala Ser Phe Gly Tyr
385 390 395 400

His Val Thr Asn Phe Phe Ala Pro Ser Ser Arg Phe Gly Thr Pro Asp
405 410 415

Asp Leu Lys Ser Leu Ile Asp Lys Ala His Glu Leu Gly Ile Val Val
420 425 430

Leu Met Asp Ile Val His Ser His Ala Ser Asn Asn Thr Leu Asp Gly
435 440 445

Leu Asn Met Phe Asp Cys Thr Asp Ser Cys Tyr Phe His Ser Gly Ala
450 455 460

Arg Gly Tyr His Trp Met Trp Asp Ser Arg Leu Phe Asn Tyr Gly Asn
465 470 475 480

Trp Glu Val Leu Arg Tyr Leu Leu Ser Asn Ala Arg Trp Trp Leu Asp
485 490 495

Ala Phe Lys Phe Asp Gly Phe Arg Phe Asp Gly Val Thr Ser Met Met
500 505 510

Tyr Ile His His Gly Leu Ser Val Gly Phe Thr Gly Asn Tyr Glu Glu
515 520 525

Tyr Phe Gly Leu Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met Leu
530 535 540

Val Asn Asp Leu Ile His Gly Leu Phe Pro Asp Ala Ile Thr Ile Gly
545 550 555 560

Glu Asp Val Ser Gly Met Pro Thr Phe Cys Ile Pro Val Gln Glu Gly
565 570 575

Gly Val Gly Phe Asp Tyr Arg Leu His Met Ala Ile Ala Asp Lys Arg
580 585 590

Ile Glu Leu Leu Lys Lys Arg Asp Glu Asp Trp Arg Val Gly Asp Ile
595 600 605

Val His Thr Leu Thr Asn Arg Arg Trp Ser Glu Lys Cys Val Ser Tyr
610 615 620

Ala Glu Ser His Asp Gln Ala Leu Val Gly Asp Lys Thr Ile Ala Phe
625 630 635 640

Trp Leu Met Asp Lys Asp Met Tyr Asp Phe Met Ala Leu Asp Arg Pro
645 650 655

Ser Thr Ser Leu Ile Asp Arg Gly Ile Ala Leu His Lys Met Ile Arg
660 665 670

Leu Val Thr Met Gly Leu Gly Gly Glu Gly Tyr Leu Asn Phe Met Gly
675 680 685

Asn Glu Phe Gly His Pro Glu Trp Ile Asp Phe Pro Arg Ala Glu Gln
690 695 700

His Leu Ser Asp Gly Ser Val Ile Pro Gly Asn Gln Phe Ser Tyr Asp
705 710 715 720

Lys Cys Arg Arg Arg Phe Asp Leu Gly Asp Ala Glu Tyr Leu Arg Tyr
725 730 735

Arg Gly Leu Gln Glu Phe Asp Arg Pro Met Gln Tyr Leu Glu Asp Lys
740 745 750

Tyr Glu Phe Met Thr Ser Glu His Gln Phe Ile Ser Arg Lys Asp Glu
755 760 765

Gly Asp Arg Met Ile Val Phe Glu Lys Gly Asn Leu Val Phe Val Phe
770 775 780

Asn Phe His Trp Thr Lys Ser Tyr Ser Asp Tyr Arg Ile Ala Cys Leu
785 790 795 800

Lys Pro Gly Lys Tyr Lys Val Ala Leu Asp Ser Asp Asp Pro Leu Phe
805 810 815

Gly Gly Phe Gly Arg Ile Asp His Asn Ala Glu Tyr Phe Thr Phe Glu
820 825 830

Gly Trp Tyr Asp Asp Arg Pro Arg Ser Ile Met Val Tyr Ala Pro Cys
835 840 845

Lys Thr Ala Val Val Tyr Ala Leu Val Asp Lys Glu Glu Glu Glu Glu
850 855 860

Glu Glu Glu Glu Glu Glu Val Ala Ala Val Glu Glu Val Val Val Glu
865 870 875 880

Glu Glu

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2576 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TCATTAAAGA GGAGAAATTA ACTATGAGAG GATCTCACCA TCACCATCAC CATGGGATCT 60

TGGCTGAAAA GTCTTCTTAC AATTCCGAAT TCCGACCTTC TACAGTTGCA GCATCGGGGA 120

AAGTCCTTGT GCCTGGAACC CAGAGTGATA GCTCCTCATC CTCAACAAAC CAATTTGAGT 180

TCACTGAGAC ATCTCCAGAA AATTCCCCAG CATCAACTGA TGTAGATAGT TCAACAATGG 240

AACACGCTAG CCAGATTAAA ACTGAGAACG ATGACGTTGA GCCGTCAAGT GATCTTACAG 300

GAAGTGTTGA AGAGCTGGAT TTTGCTTCAT CACTACAACT ACAAGAAGGT GGTAAACTGG 360

AGGAGTCTAA AACATTAAAT ACTTCTGAAG AGACAATTAT TGATGAATCT GATAGGATCA 420

GAGAGAGGGG CATCCCTCCA CCTGGACTTG GTCAGAAGAT TTATGAAATA GACCCCCTTT 480

TGACAAACTA TCGTCAACAC CTTGATTACA GGTATTCACA GTACAAGAAA CTGAGGGAGG 540

CAATTGACAA GTATGAGGGT GGTTTGGAAG CTTTTTCTCG TGGTTATGAA AAAATGGGTT 600

TCACTCGTAG TGCTACAGGT ATCACTTACC GTGAGTGGGC TCCTGGTGCC CAGTCAGCTG 660

CCCTCATTGG AGATTTCAAC AATTGGGACG CAAATGCTGA CATTATGACT CGGAATGAAT 720

TTGGTGTCTG GGAGATTTTT CTGCCAAATA ATGTGGATGG TTCTCCTGCA ATTCCTCATG 780

GGTCCAGAGT GAAGATACGT ATGGACACTC CATCAGGTGT TAAGGATTCC ATTCCTGCTT 840

GGATCAACTA CTCTACAGCT TCCTGATGAA ATTCCATATA ATGGAATATA TTATGATCCA 900

CCCGAAGAGG AGAGGTATAT CTTCCAACAC CCACGGCCAA AGAAACCAAA GTCGCTGAGA 960

ATATATGAAT CTCATATTGG AATGAGTAGT CCGGAGCCTA AAATTAACTC ATACGTGAAT 1020

TTTAGAGATG AAGTTCTTCC TCGCATAAAA AAGCTTGGGT ACAATGCGCT GCAAATTATG 1080

GCTATTCAAG AGCATTCTTA TTATGCTAGT TTTGGTTATC ATGTCACAAA TTTTTTTGCA 1140

CCAAGCAGCC GTTTTGGAAC GCCCGACGAC CTTAAGTCTT TGATTGATAA AGCTCATGAG 1200

CTAGGAATTG TTGTTCTCAT GGACATTGTT CACAGCCATG CATCAAATAA TACTTTAGAT 1260

GGACTGAACA TGTTTGACGG CACCGATAGT TGTTACTTTC ACTCTGGAGC TCGTGGTTAT 1320

CATTGGATGT GGGATTCCCG CCTTTTTAAC TATGGAAACT GGGAGGTACT TAGGTATCTT 1380

CTCTCAAATG CGAGATGGTG GTTGGATGAG TTCAAATTTG ATGGATTTAG ATTTGATGGT 1440

GTGACATCAA TGATGTATAC TCACCACGGA TTATCGGTGG GATTCACTGG GAACTACGAG 1500

GAATACTTTG GACTCGCAAC TGATGTGGAT GCTGTTGTGT ATCTGATGCT GGTCAACGAT 1560

CTTATTCATG GGCTTTTCCC AGATGCAATT ACCATTGGTG AAGATGTTAG CGGAATGCCG 1620

ACATTTTGTA TTCCCGTTCA AGATGGGGGT GTTGGCTTTG ACTATCGGCT GCATATGGCA 1680

ATTGCTGATA AATGGATTGA GTTGCTCAAG AAACGGGATG AGGATTGGAG AGTGGGTGAT 1740

ATTGTTCATA CACTGACAAA TAGAAGATGG TCGGAAAAGT GTGTTTCATA CGCTGAAAGT 1800

CATGATCAAG CTCTAGTCGG TGATAAAACT ATAGCATTCT GGCTGATGGA CAAGGATATG 1860

TATGATTTTA TGGCTCTGGA TAGACCGCCA ACATCATTAA TAGATCGTGG GATAGCATTG 1920

CACAAGATGA TTAGGCTTGT AACTATGGGA TTAGGAGGAG AAGGGTACCT AAATTTCATG 1980

GGAAATGAAT TCGGCCACCC TGAGTGGATT GATTTCCCTA GGGCTGAACA ACACCTCTCT 2040

GATGACTCAG TAATTCCCGG AAACCAATTC AGTTATGATA AATGCAGACG GAGATTTGAC 2100

CTGGGAGATG CAGAATATTT AAGATACCGT GGGTTGCAAG AATTTGACCG GGCTATGCAG 2160

TATCTTGAAG ATAAATATGA GTTTATGACT TCAGAACACC AGTTCATATC ACGAAAGGAT 2220

GAAGGAGATA GGATGATTGT ATTTGAAAAA GGAAACCTAG TTTTTGTCTT TAATTTTCAC 2280

TGGACAAAAA GCTATTCAGA CTATCGCATA GGCTGCCTGA AGCCTGGAAA ATACAAGGTT 2340

GCCTTGGACT CAGATGATCC ACTTTTTGGT GGCTTCGGGA GAATTGATCA TAATGCCGAA 2400

TATTTCACCT TTGAAGGATG GTATGATGAT CGTCCTCGTT CAATTATGGT GTATGCACCT 2460

TGTAGAACAG CAGTGGTCTA TGCACTAGTA GACAAAGAAG AAGAAGAAGA AGAAGAAGAA 2520

GAAGAAGTAG CAGTAGTAGA AGAAGTAGTA GTAGAAGAAG AATGAACGAA CTTGTG 2576

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2529 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

GGATGCTAAT GTTTCTGTAT TCTTGAAAAA GCACTCTCTT TCACGGAAGA TCTTGGCTGA 60

AAAGTCTTCT TACAATTCCG AATCCCGACC TTCTACAGTT GCAGCATCGG GGAAAGTCCT 120

TGTGCCTGGA AYCCAGAGTG ATAGCTCCTC ATCCTCAACA GACCAATTTG AGTTCACTGA 180

GACATCTCCA GAAAATTCCC CAGCATCAAC TGATGTAGAT AGTTCAACAA TGGAACACGC 240

TAGCCAGATT AAAACTGAGA ACGATGACGT TGAGCCGTCA AGTGATCTTA CAGGAAGTGT 300

TGAAGAGCTG GATTTTGCTT CATCACTACA ACTACAAGAA GGTGGTAAAC TGGAGGAGTC 360

TAAAACATTA AATACTTCTG AAGAGACAAT TATTGATGAA TCTGATAGGA TCAGAGAGAG 420

GGGCATCCCT CCACCTGGAC TTGGTCAGAA GATTTATGAA ATAGACCCCC TTTTGACAAA 480

CTATCGTCAA CACCTTGATT ACAGGTATTC ACAGTACAAG AAACTGAGGG AGGCAATTGA 540

CAAGTATGAG GGTGGTTTGG AAGCTTTTTC TCGTGGTTAT GAAAAAATGG GTTTCACTCG 600

TAGTGCTACA GGTATCACTT ACCGTGAGTG GGCTCCTGGT GCCCAGTCAG CTGCCCTCAT 660

TGGAGATTTC AACAATTGGG ACGCAAATGC TGACATTATG ACTCGGAATG AATTTGGTGT 720

CTGGGAGATT TTTCTGCCAA ATAATGTGGA TGGTTCTCCT GCAATTCCTC ATGGGTCCAG 780

AGTGAAGATA CGYATGGACA CTCCATCAGG TGTTAAGGAT TCCATTCCTG CTTGGATCAA 840

CTACTCTTTA CAGCTTCCTG ATGAAATTCC ATATAATGGA ATATATTATG ATCCACCCGA 900

AGAGGAGAGG TATRTCTTCC AACACCCACG GCCAAAGAAA CCAAAGTCGC TGAGAATATA 960

TGAATCTCAT ATTGGAATGA GTAGTCCGGA GCCTAAAATT AACTCATACG TGAATTTTAG 1020

AGATGAAGTT CTTCCTCGCA TAAAAAASCT TGGGTACAAT GCGGTGCAAA TTATGGCTAT 1080

TCAAGAGCAT TCTTATTATG CTAGTTTTGG TTATCATGTC ACAAATTTTT TTGCACCAAG 1140

CAGCCGTTTT GGAACGCCCG ACGACCTTAA GTCTTTGATT GATAAAGCTC ATGAGCTAGG 1200

AATTGTTGTT CTCATGGACA TTGTTCACAG CCATGCATCA AATAATACTT TAGATGGACT 1260

GAACATGTTT GACGGCACAG ATAGTTGTTA CTTTCACTCT GGAGCTCGTG GTTATCATTG 1320

GATGTGGGAT TCCCGCCTCT TTAACTATGG AAACTGGGAG GTACTTAGGT ATCTTCTCTC 1380

AAATGCGAGA TGGTGGTTGG ATGAGTTCAA ATTTGATGGA TTTAGATTTG ATGGTGTGAC 1440

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CTTTGGACTC GCAACTGATG TGGATGCTGT TGTGTATCTG ATGCTGGTCA ACGATCTTAT 1560

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CACCTCTGAA GGATCGTATG ATGATCGTCC TCGTTCAATT ATGGTGTATG CACCTAGTAG 2460

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NGAAGAATT 2529

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3231 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

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ACACTCAGTT AGTTACACTC CTATCACTCA TCAGATCTCT ATTTTTTCTC TTAATTCCAA 180

CCAAGGAATG AATTAAAAGA TTAGATTTGA AGGAGAGAAG AAGAAAGATG GTGTATACAC 240

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AACTGGAGGA GTCTAAAACA TTAAATACTT CTGAAGAGAC AATTATTGAT GAATCTGATA 720

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TGGGTTTCAC TCGTAGTGCT ACAGGTATCA CTTACCGTGA GTGGGCTCCT GGTGCCCAGT 960

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ATGAATTTGG TGTCTGGGAG ATTTTTCTGC CAAATAATGT GGATGGTTCT CCTGCAATTC 1080

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(2) INFORMATION FOR SEQ ID NO: 19:

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(A) LENGTH: 2578 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

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CTTGTAGAAC AGCAGTGGTC TATGCACTAG TAGACAAAGA AGAAGAAGAA GAAGAAGAAG 2520

AAGAAGAAGT AGCAGTAGTA GAAGAAGTAG TAGTAGAAGA AGAATGAACG AACTTGTG 2578

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

AATTTYATGG GNAAYGARTT YGG 23

CLAIMS

1. Starch extracted from a potato plant and having an amylose content of at least 35%, as judged by the iodometric assay method of Morrison & Laignelet (1983 J. Cereal Science *1*, 9-20).

2. Starch according to claim 1, having an amylose content of at least 37%, as judged by the method defined in claim 1.

3. Starch according to claim 1, having an amylose content of at least 40%, as judged by the method defined in claim 1.

4. Starch according to claim 1, having an amylose content of at least 50%, as judged by the method defined in claim 1.

5. Starch according to claim 1, having an amylose content of at least 66%, as judged by the method defined in claim 1.

6. Starch according to any one of claims 1-5, having an amylose content of 35 - 66%, as judged by the method defined in claim 1.

7. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity onset temperature in the range 70 - 95°C, as judged by viscoamylograph of a 10% w/w aqueous suspension thereof, performed at atmospheric pressure using the Newport Scientific Rapid Visco Analyser 3C with a heating profile of holding at 50°C for 2 minutes, heating from 50 to 95°C at a rate of 1.5°C per minute, holding at 95°C for 15 minutes, cooling from 95 to 50°C at a rate of 1.5°C per minute, and then holding at 50°C for 15 minutes.

8. Starch which as extracted from a potato plant by wet milling at ambient temperature has peak viscosity in the range 500 - 12 stirring number units (SNUs), as judged by viscoamylograph conducted according to the protocol defined in claim 7.

9. Starch which as extracted from a potato plant by wet milling at ambient temperature has a pasting viscosity in the range 214 - 434 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

10. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 450 - 618 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

11. Starch which as extracted from a potato plant by wet milling at ambient temperature has a set-back viscosity in the range 14 - 192 SNUs, as judged by viscoamylograph conducted according to the protocol defined in claim 7.

12. Starch which as extracted from a potato plant by wet milling at ambient temperature has a peak viscosity in the range 200 - 500 SNUs and a set-back viscosity in the range 275-618 SNUs as judged by viscoamylograph according to the protocol defined in claim 7.

13. Starch which as extracted from a potato plant by wet milling at ambient temperature has a viscosity which does not decrease between the start of the heating phase (step 2) and the start of the final holding phase (step 5) and has a set-back viscosity of 303 SNUs or less as judged by viscoamylograph according to the protocol defined in claim 7.

14. Starch which as extracted from a potato plant by wet milling at ambient temperature displays no significant increase in viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7.

15. Starch which as extracted from a potato plant by wet milling at ambient temperature, is in accordance with claim 7 and in accordance with any one of claims 8 to 14.

16. Starch according to any one of claims 7 to 15, having an amylose content in the range 35 - 66%, as judged by the method of Morrison & Laignelet defined in claim 1.

17. Starch which as extracted from a potato plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.

18. Starch according to claim 17, having a phosphorus content in the range 200 - 240mg/100grams dry weight starch.

19. Starch according to claim 17 or 18, further in accordance with any one of claims 1 to 16.

20. Starch prepared by physical, chemical and/or enzymatic treatment of a starch initially having properties in accordance with any one of claims 1-19.

21. Starch according to claim 20, being resistant starch prepared by physical, chemical and/or enzymatic treatment of a starch initially having properties in accordance with any one of claims 1-19.

22. Starch according to claim 21, comprising in excess of 5% total dietary fibre, as determined according to the method of Prosky *et al.*, (1985 J. Assoc. Off. Anal. Chem. *68*, 677).

23. Use of starch according to any one of claims 1-22 in the preparation or processing of a foodstuff.

24. Use of starch according to claim 23, wherein the starch is used to provide a film, barrier, coating or as a gelling agent.

25. Use of starch according to claim 23, to prepare resistant starch compositions.

26. Use of starch according to any one of claims 1-22 in the preparation or processing of corrugating adhesives, biodegradable products, packaging, glass fibers and textiles.

27. A nucleotide sequence encoding an effective portion of a class A starch branching

enzyme (SBE) obtainable from potato plants.

28. A nucleotide sequence according to claim 27, encoding a polypeptide comprising substantially the amino acid sequence of residues 49 to 882 of the sequence shown in Figure 5.

29. A nucleotide sequence according to claim 27 or 28, comprising substantially the sequence of nucleotides 289 to 2790 of the sequence shown in Figure 5, or a functional equivalent thereof.

30. A nucleotide sequence according to claim 29, further comprising the sequence of nucleotides 145 to 288 of the sequence shown in Figure 5, or a functional equivalent thereof.

31. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 228 to 2855 of the sequence labelled psbe2con.seq in Figure 8, or a functional equivalent thereof.

32. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 57 to 2564 of the sequence labelled as psbe2con.seq in Figure 12, or a functional equivalent thereof.

33. A nucleotide sequence according to any one of claims 27 to 32, comprising an in-frame ATG start codon, and optionally including a 5' and/or a 3' untranslated region.

34. A nucleotide sequence according to claim 27, comprising the sequence of nucleotides 45 to 3200 of the sequence labelled as psbe2con.seq in Figure 8, or a functional equivalent thereof.

35. A nucleic acid construct comprising a sequence in accordance with any one of claims 27 to 34.

36. An expression vector comprising a nucleic acid construct according to claim 35.

37. A host cell into which has been introduced a sequence in accordance with any one of claims 27 to 36.

38. An effective portion of a class A SBE polypeptide obtainable from potato plants and encoded by a nucleotide sequence in accordance with any one of claims 27 to 36.

39. A polypeptide according to claim 38, comprising substantially the sequence of amino acids 49 to 882 of the sequence shown in Figure 5, or a functional equivalent thereof.

40. A polypeptide according to claim 38 or 39, comprising the sequence of amino acids 1 to 48 of the sequence shown in Figure 5.

41. A polypeptide in accordance with any one of claims 38, 39 or 40 in substantial isolation from other plant-derived constituents.

42. A method of altering the characteristics of a plant, comprising introducing into the plant a portion of a nucleotide sequence in accordance with any one of claims 27 to 36, operably linked to a suitable promoter active in the plant, so as to affect the expression of a gene present in the plant.

43. A method according to claim 42, wherein the nucleotide sequence is operably linked in the anti-sense orientation to a suitable promoter active in the plant.

44. A method according to claim 42, wherein the introduced sequence comprises one or more of the following operably linked in the sense orientation to a promoter active in the plant, so as to cause sense suppression of an enzyme naturally expressed in the plant: a 5' untranslated region, a 3' untranslated region, or a coding region of the potato SBE class A starch branching enzyme.

45. A method according to any one of claims 42, 43 or 44, further comprising

introducing into the plant one or more further sequences.

46. A method according to claim 45, wherein one or more of the further sequences are operably linked in the anti-sense orientation to a suitable promoter active in the plant.

47. A method according to claim 45 or 46, wherein the further sequence comprises a portion of a class B SBE nucleotide sequence.

48. A method according to any one of claims 42 to 47, effective in altering the starch composition of a plant.

49. A plant or plant cell having characteristics altered by the method of any one of claims 42 to 48, or the progeny of such a plant, or part of such a plant.

50. A plant according to claim 49, selected from one of the following: potato, pea, tomato, maize, wheat, rice, barley, sweet potato, and cassava.

51. A tuber or other storage organ from a plant according to claim 49 or 50.

52. Use of a tuber or other storage organ according to claim 51, in the preparation and/or processing of a foodstuff.

53. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated viscosity onset temperature as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

54. A plant according to claim 53, wherein the viscosity onset temperature is elevated by an amount in the range of 10 to 25°C.

55. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased peak viscosity as judged by

viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

56. A plant according to claim 55, wherein the peak viscosity is decreased by an amount in the range of 240 to 700 SNUs.

57. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an increased pasting viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

58. A plant according to claim 57, wherein the pasting viscosity is increased by an amount in the range of 37 to 260 SNUs.

59. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an increased set-back viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

60. A plant according to claim 59, wherein the set-back viscosity is increased by an amount in the range of 224 to 313 SNUs.

61. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has a decreased set-back viscosity as judged by viscoamylograph conducted according to the protocol defined in claim 7, compared to starch extracted from a similar, but unaltered, plant.

62. A plant according to claim 49 or 50, containing starch which, as extracted from the plant by wet milling at ambient temperature, has an elevated apparent amylose content as judged by iodometric assay according to the method of Morrison & Laignelet, compared to starch extracted from a similar, but unaltered, plant.

63. A plant according to claim 49 or 50, containing starch which, as extracted from the plant, has a phosphorus content in excess of 200mg/100grams dry weight starch.

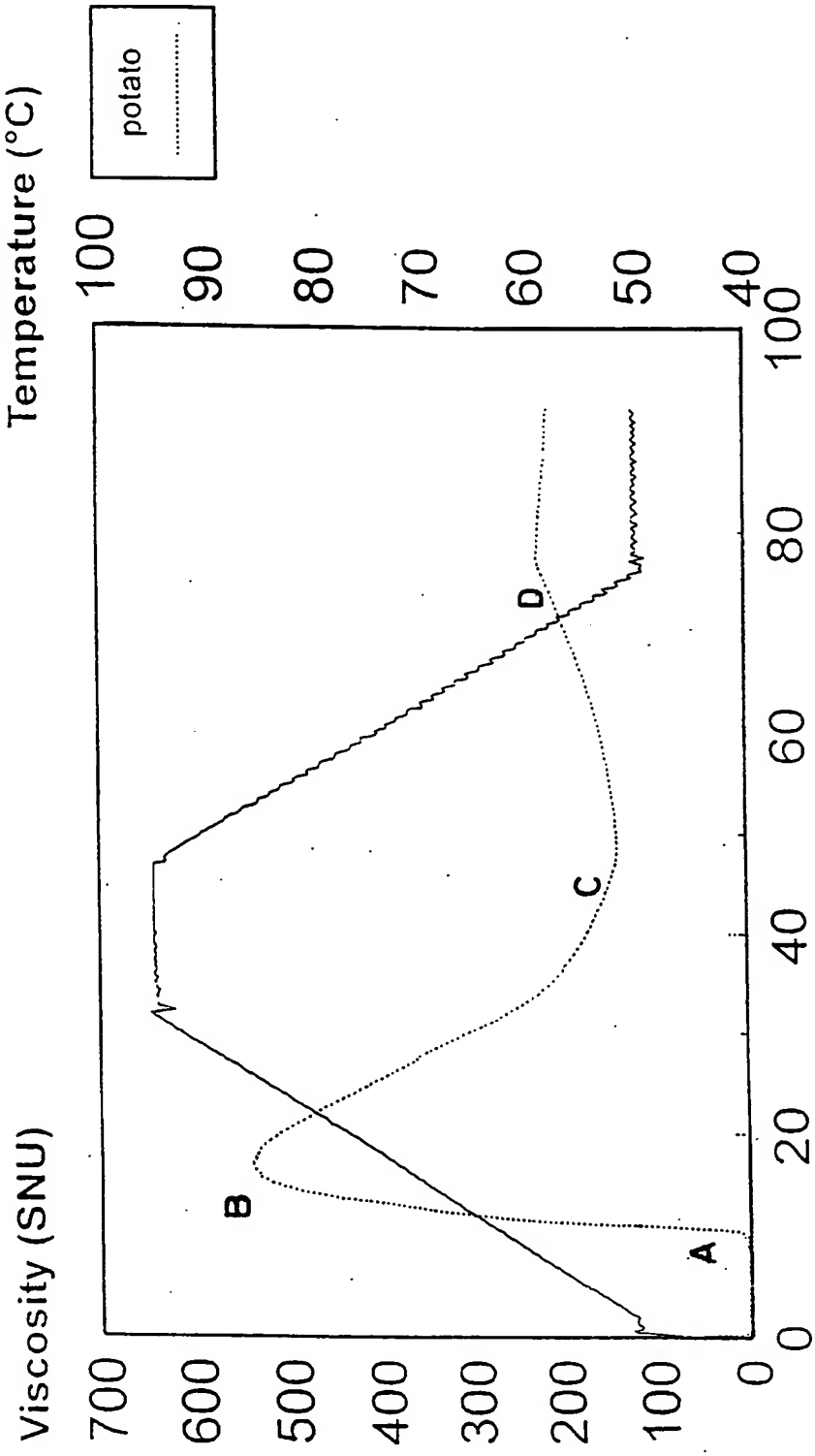
64. Starch obtainable from a plant according to any one of claims 49, 50 or 53 - 63.

65. Starch according to claim 64 and further in accordance with any one of claims 1 - 22.

66. A method of modifying starch *in vitro*, comprising treating starch under suitable conditions with an effective amount of a polypeptide in accordance with any one of claims 38 to 41.

67. A potato plant or part thereof which, in its wild type possesses an effective SBE A gene, but which plant has been altered such that there is no effective expression of an SBE A polypeptide within the cells of at least part of the plant.

68. A potato plant according to claim 67, wherein the alteration is effected by a method according to any one of claims 42-48.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200
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40
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100
Time (min)
Temperature (°C)
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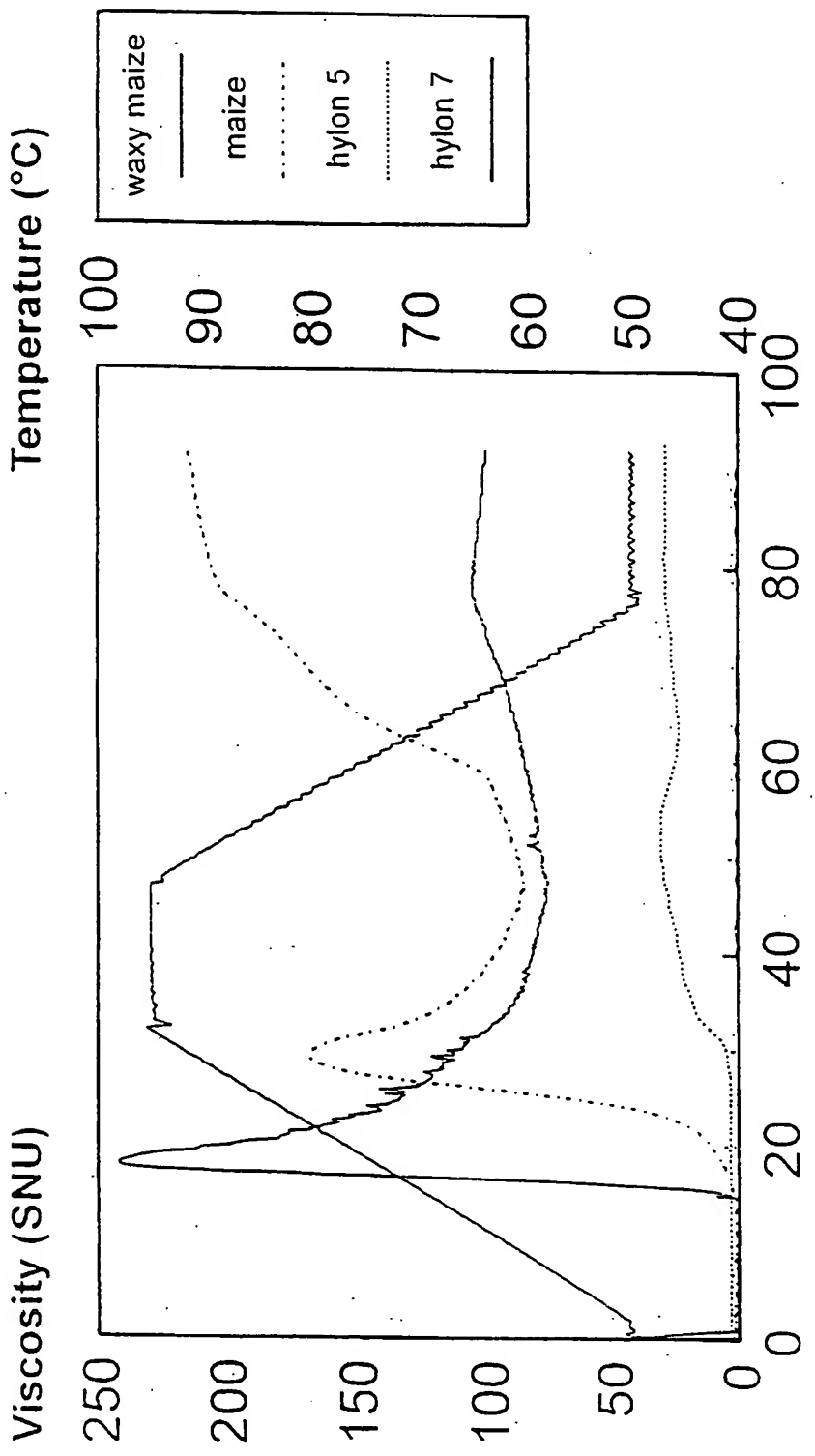
90
80
70
60
50
40
potato
A
B
C
D

Fig. 1


Viscosity (SNU)
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200
150
100
50
0
Temperature (°C)
100
90
80
70
60
50
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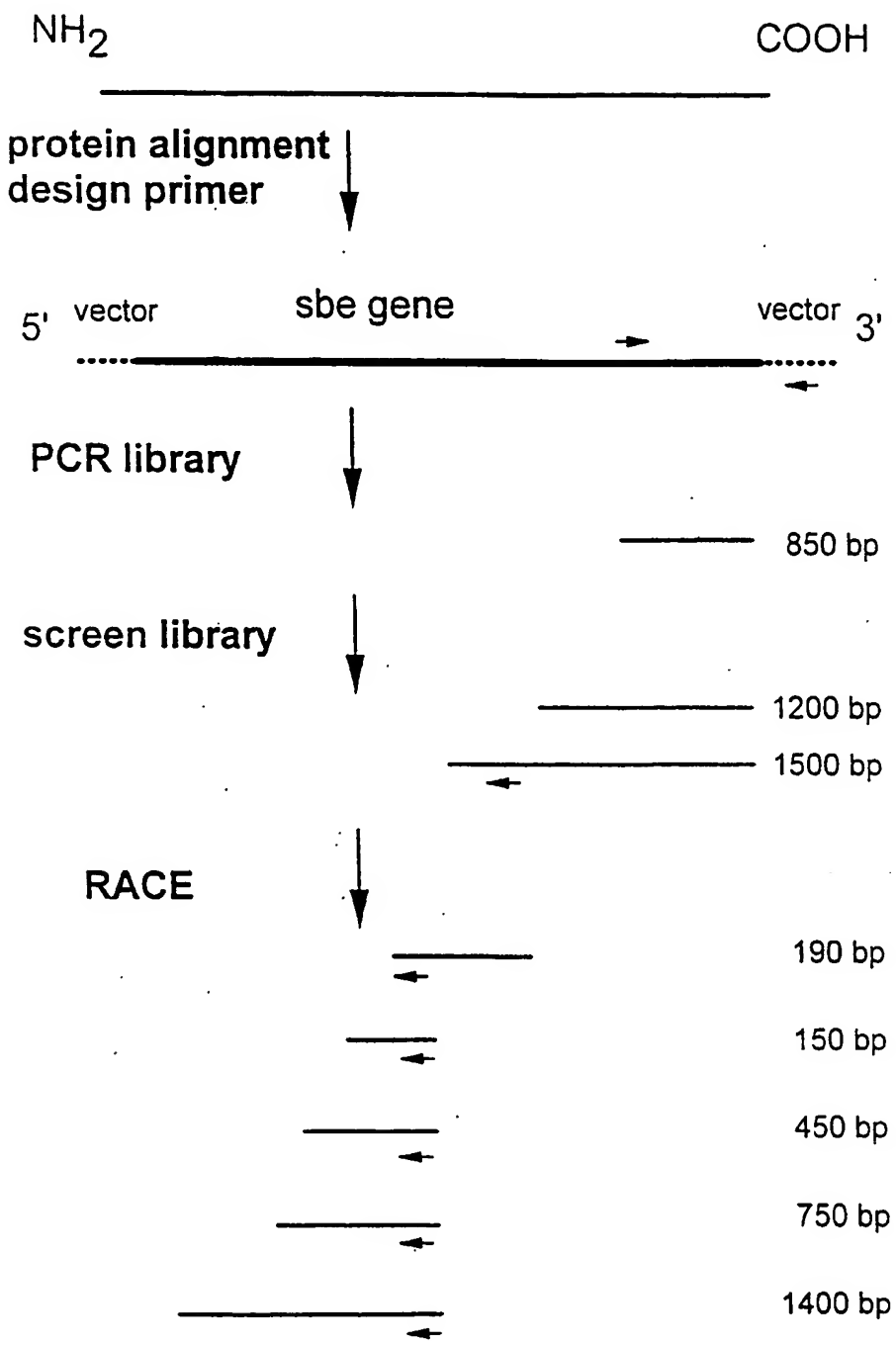
20
40
60
80
100
Time (min)
waxy maize
maize
hylon 5
hylon 7

Fig. 2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vector
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Fig. 3

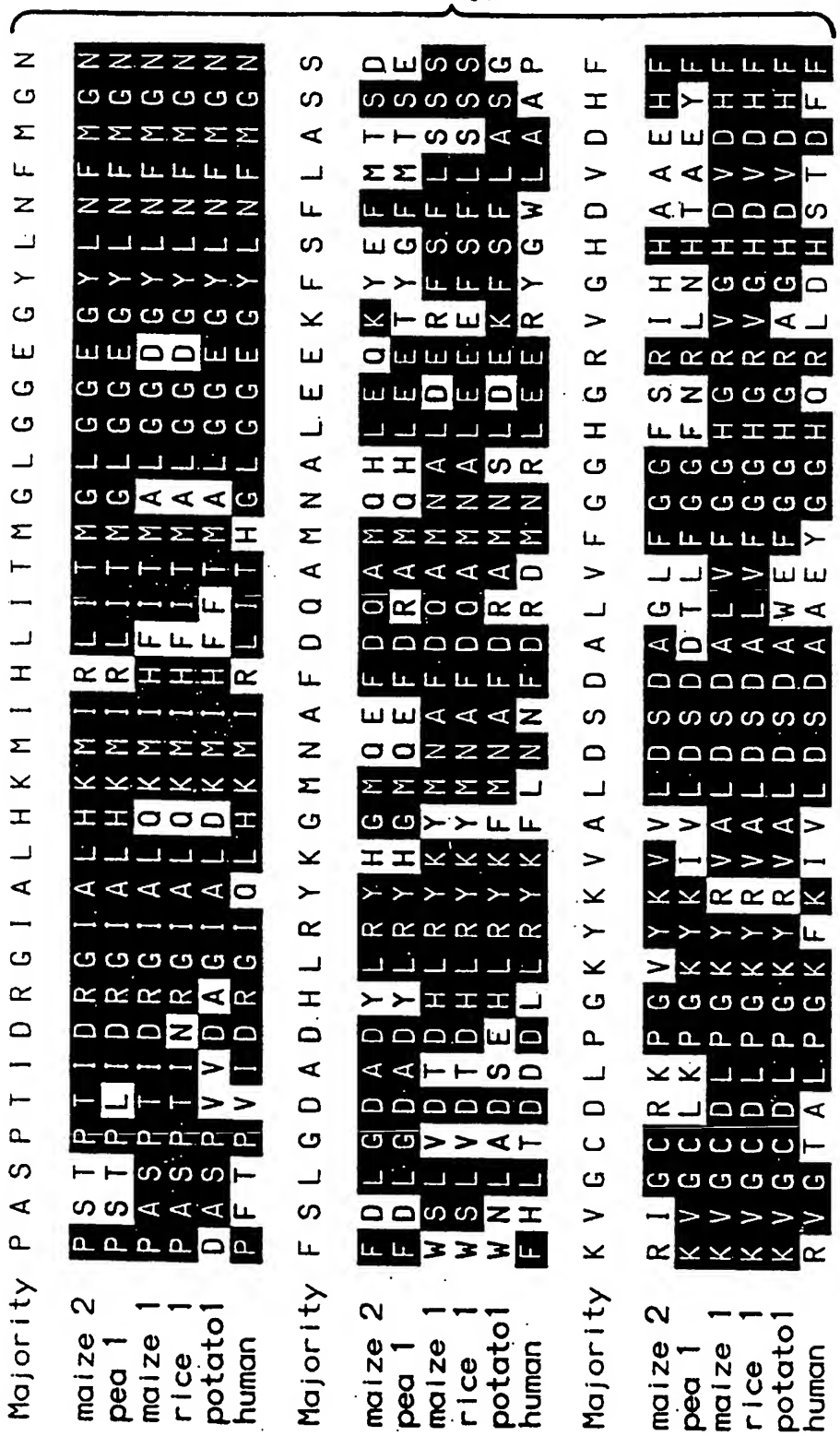

Fig. 4a SHEET 1

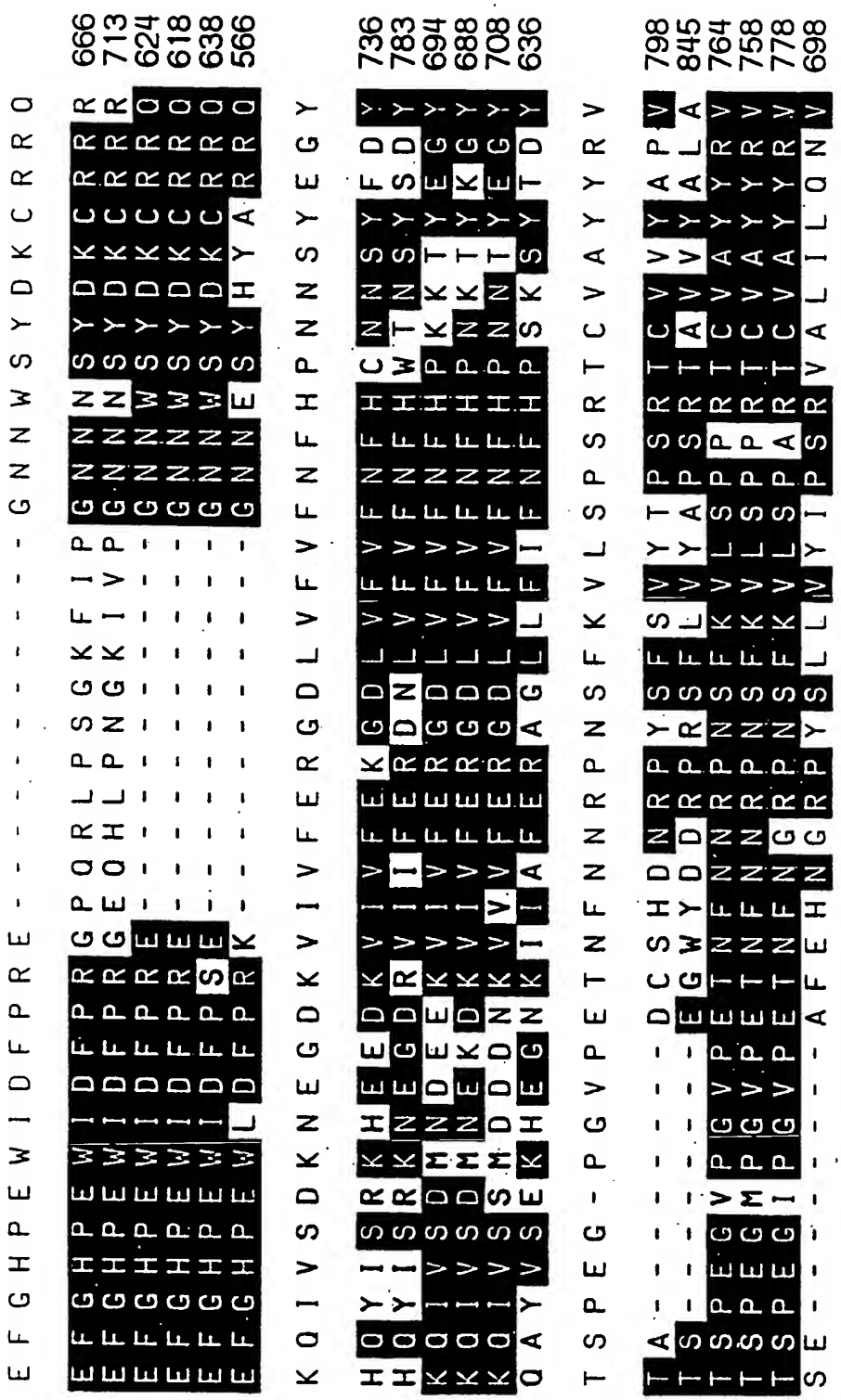

Fig. 4a SHEET 2

```
Majority D E X X S E - R - - - - - - - - - - - - - - - - - - - - - - - - - - -

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maize 1  D E A G A G R R L H A K A E T G K T S P A E S I D V K A S R A S S K
rice  1  D E D R E E L R R G G
potato1  D E R M S E T E D Y Q
human    D L P N .

- - - - - - - - - - - - - - - - - - - - - - - - -

E D K E A T A G G K K G W K F A R Q P S D Q D T K .          800
                                                              856
                                                              824
                                                              769
                                                              789
                                                              703
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Fig. 4a SHEET 3

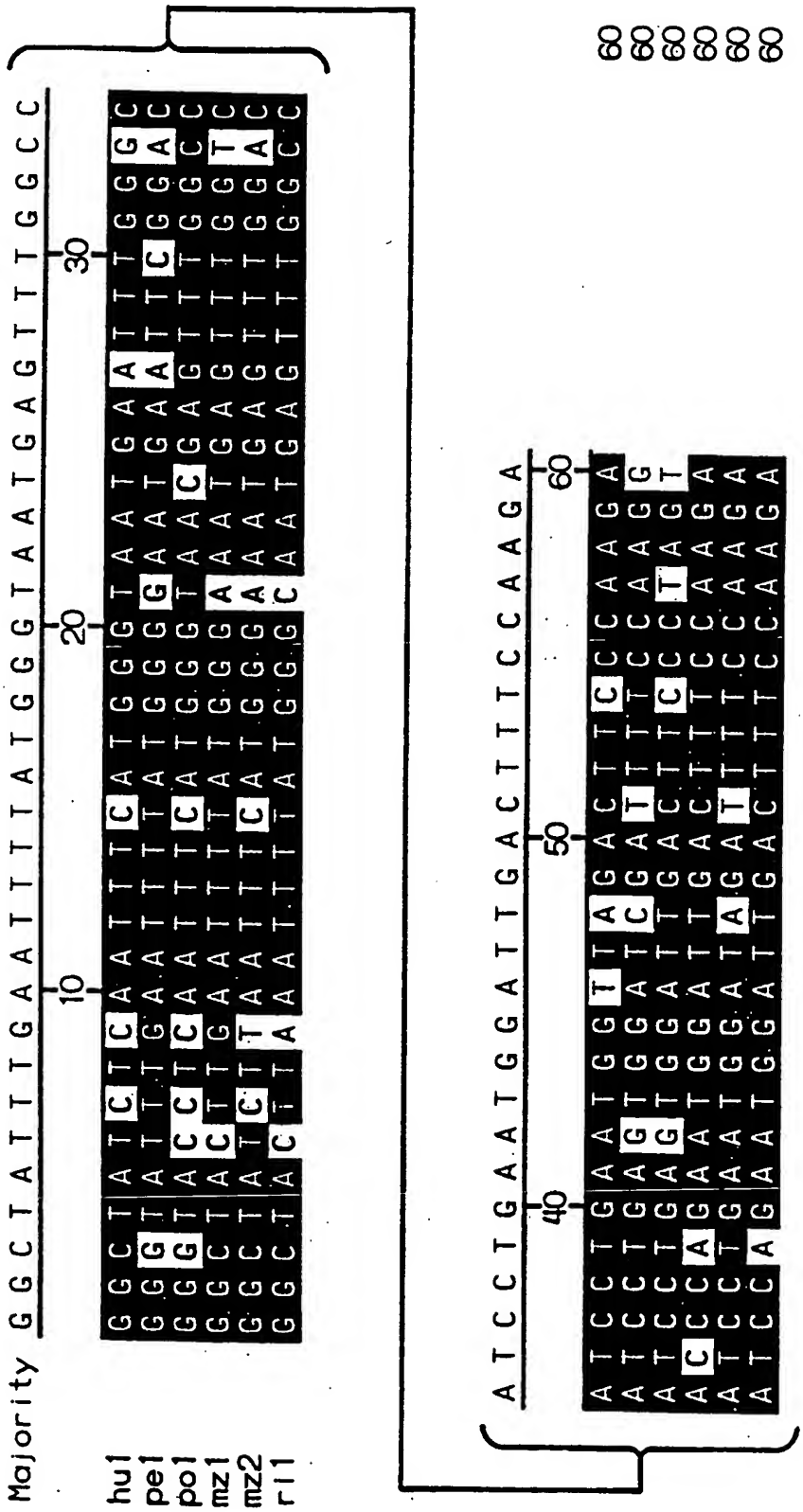


Fig. 4b

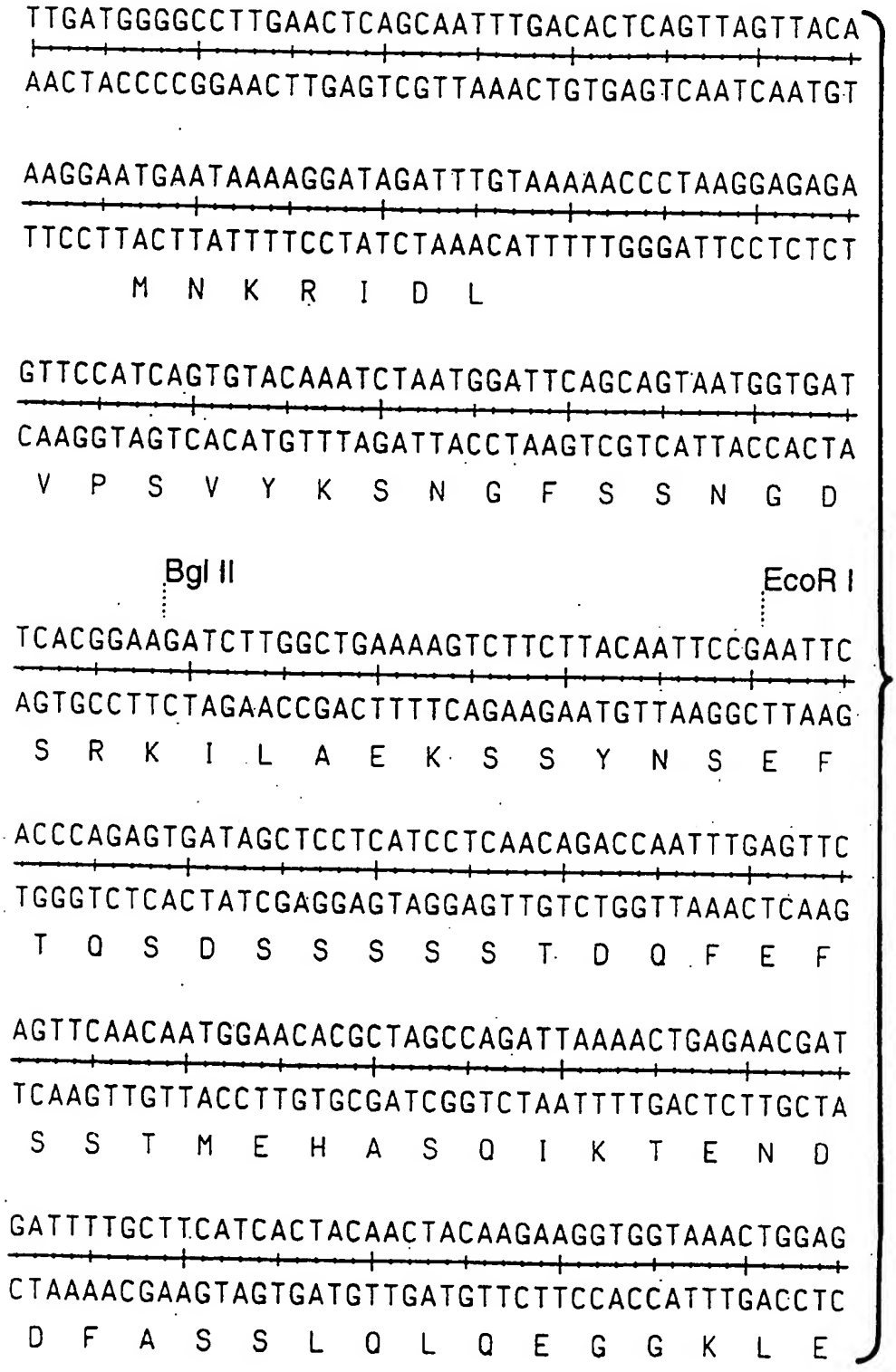
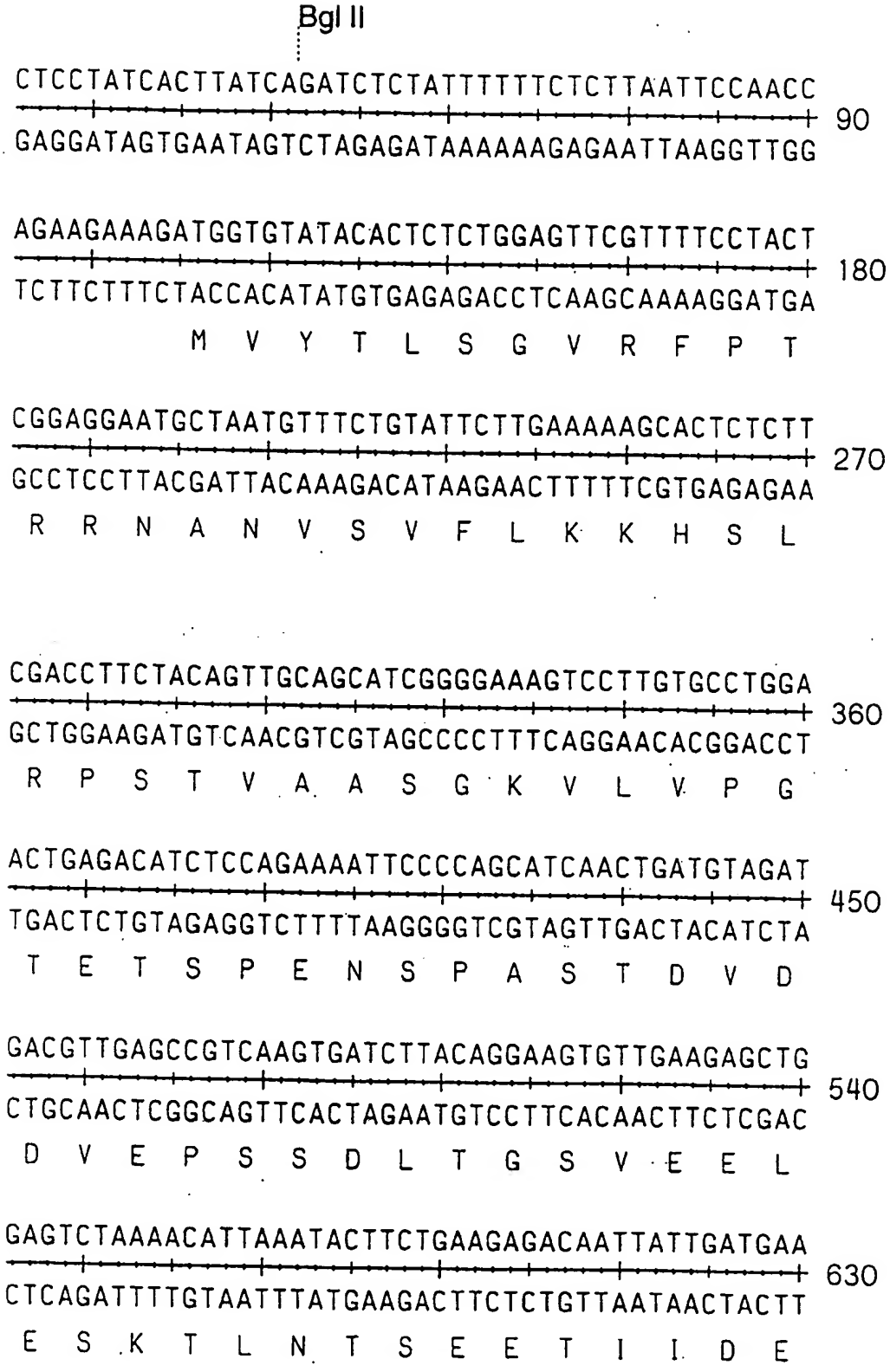


Fig 5 Sheet 2

Fig. 5 SHEET 1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TCTTCTTTCTACCACATATGTGAGAGACCTCAAGCAAAAGGATGA
180
M V Y T L S G V R F P T
CGGAGGAATGCTAATGTTTCTGTATTCTTGAAAAAGCACTCTCTT
GCCTCCTTACGATTACAAAGACATAAGAACTTTTTCGTGAGAGAA
270
R R N A N V S V F L K K H S L
CGACCTTCTACAGTTGCAGCATCGGGGAAAGTCCTTGTGCCTGGA
GCTGGAAGATGTCAACGTCGTAGCCCCTTTCAGGAACACGGACCT
360
R P S T V A A S G K V L V P G
ACTGAGACATCTCCAGAAAATTCCCCAGCATCAACTGATGTAGAT
TGACTCTGTAGAGGTCTTTTAAGGGGTCGTAGTTGACTACATCTA
450
T E T S P E N S P A S T D V D
GACGTTGAGCCGTCAAGTGATCTTACAGGAAGTGTTGAAGAGCTG
CTGCAACTCGGCAGTTCACTAGAATGTCCTTCACAACTTCTCGAC
540
D V E P S S D L T G S V E E L
GAGTCTAAAACATTAAATACTTCTGAAGAGACAATTATTGATGAA
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630
E S K T L N T S E E T I I D E

Fig 5 SHEET 2

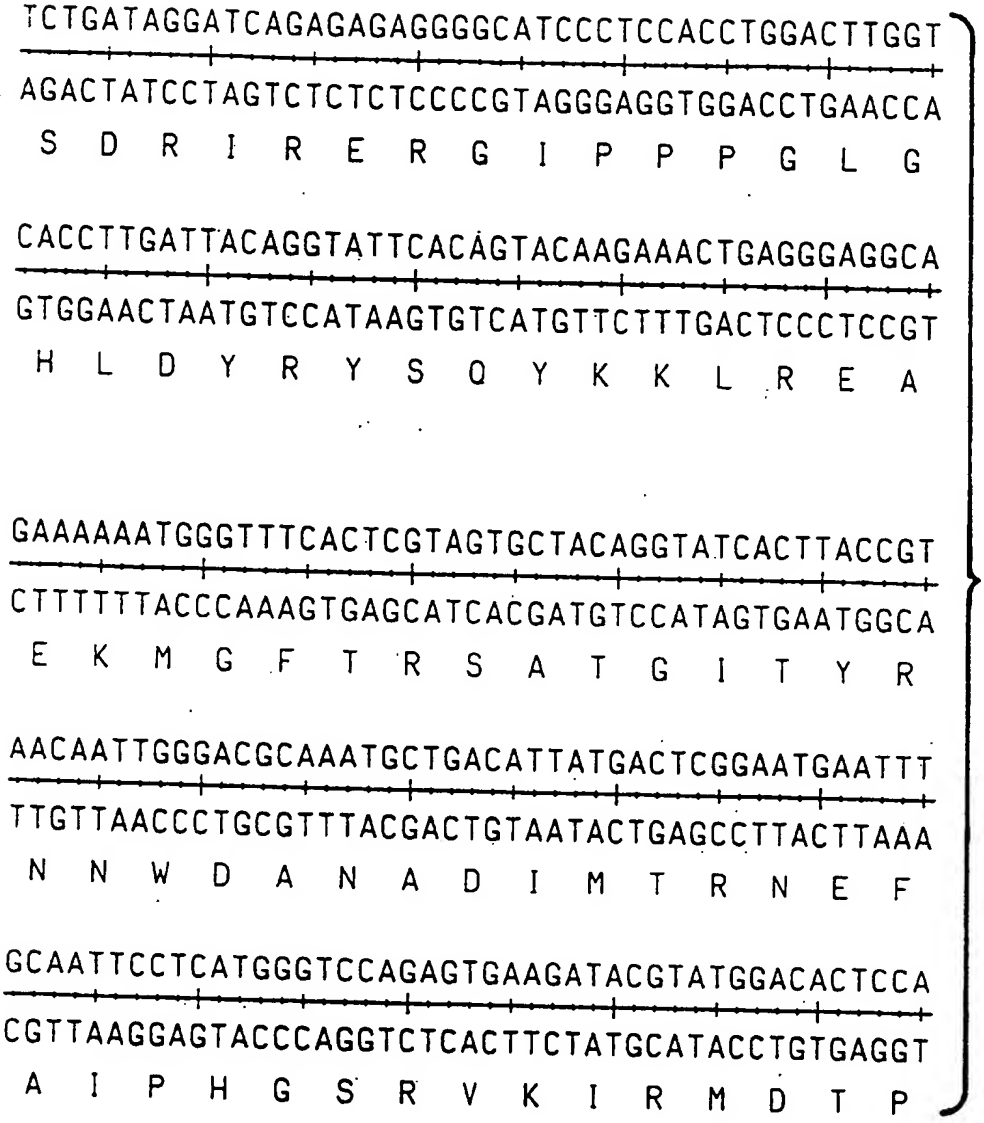
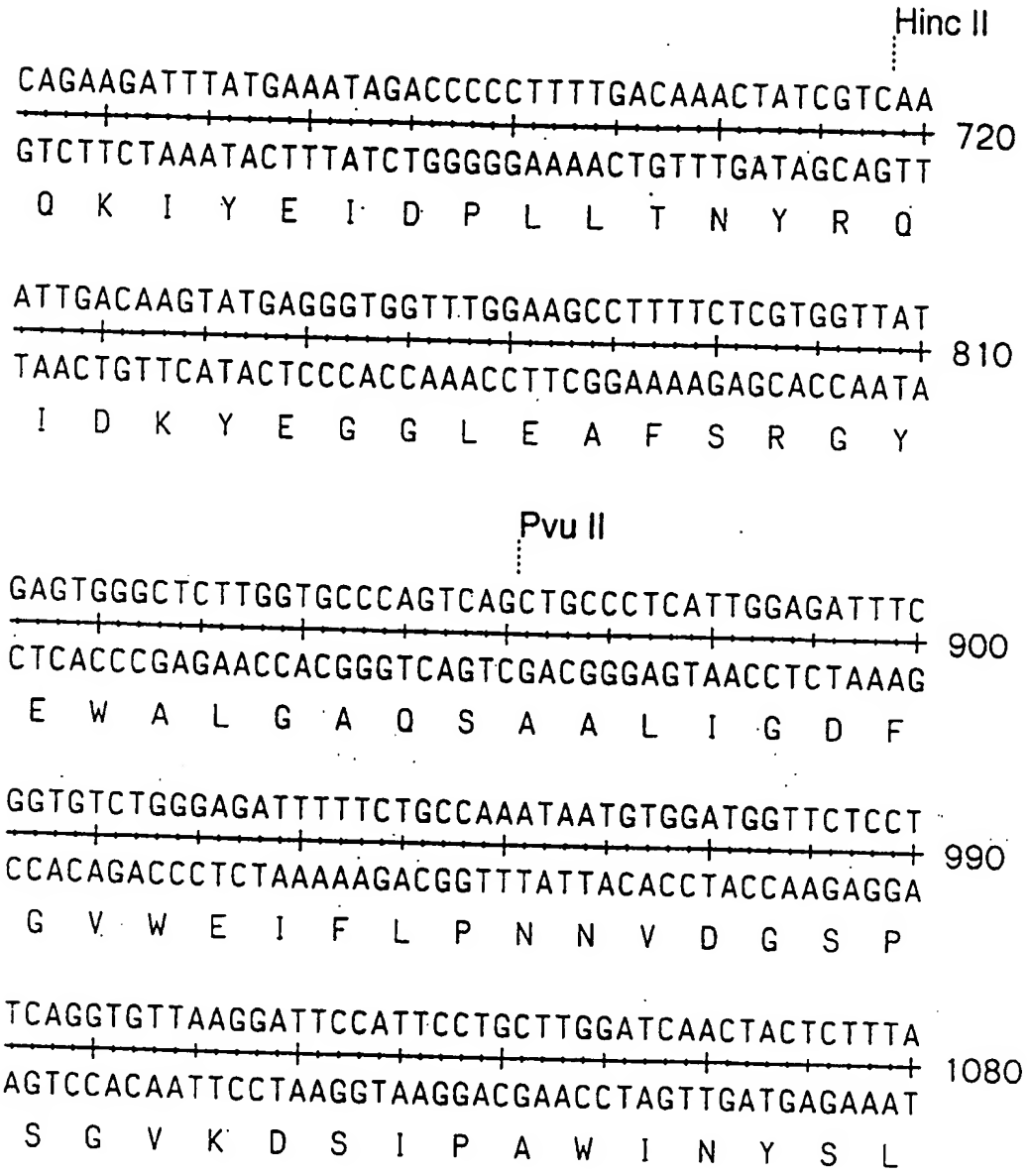


Fig.5 Sheet 4

Fig. 5 SHEET 3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720
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I D K Y E G G L E A F S R G Y
810
Pvu II
GAGTGGGCTCTTGGTGCCCAGTCAGCTGCCCTCATTGGAGATTTC
CTCACCCGAGAACCACGGGTCAGTCGACGGGAGTAACCTCTAAAG
E W A L G A Q S A A L I G D F
900
GGTGTCTGGGAGATTTTTCTGCCAAATAATGTGGATGGTTCTCCT
CCACAGACCCTCTAAAAAGACGGTTTATTACACCTACCAAGAGGA
G V W E I F L P N N V D G S P
990
TCAGGTGTTAAGGATTCCATTCCTGCTTGGATCAACTACTCTTTA
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1080

Fig. 5 SHEET 4

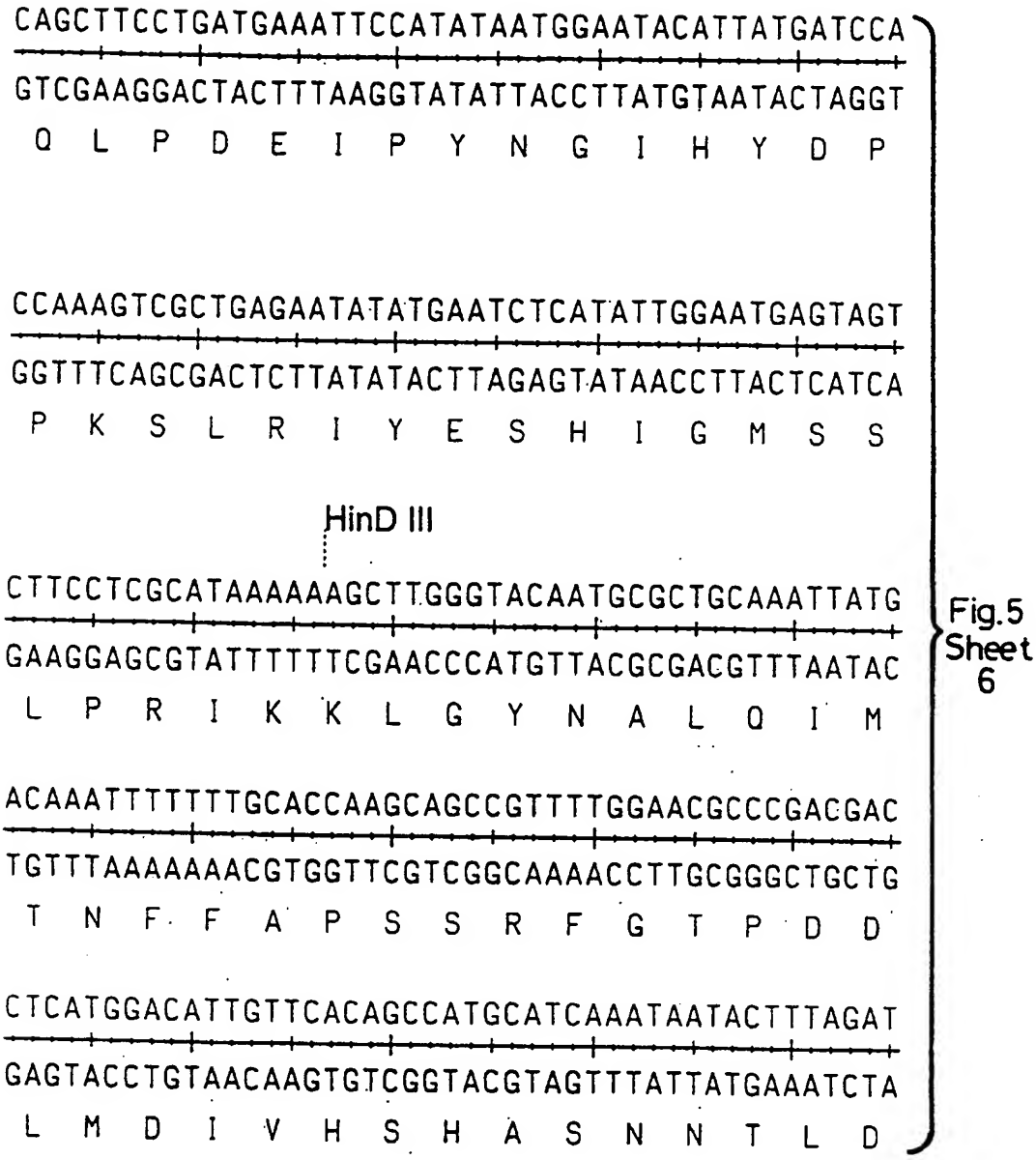
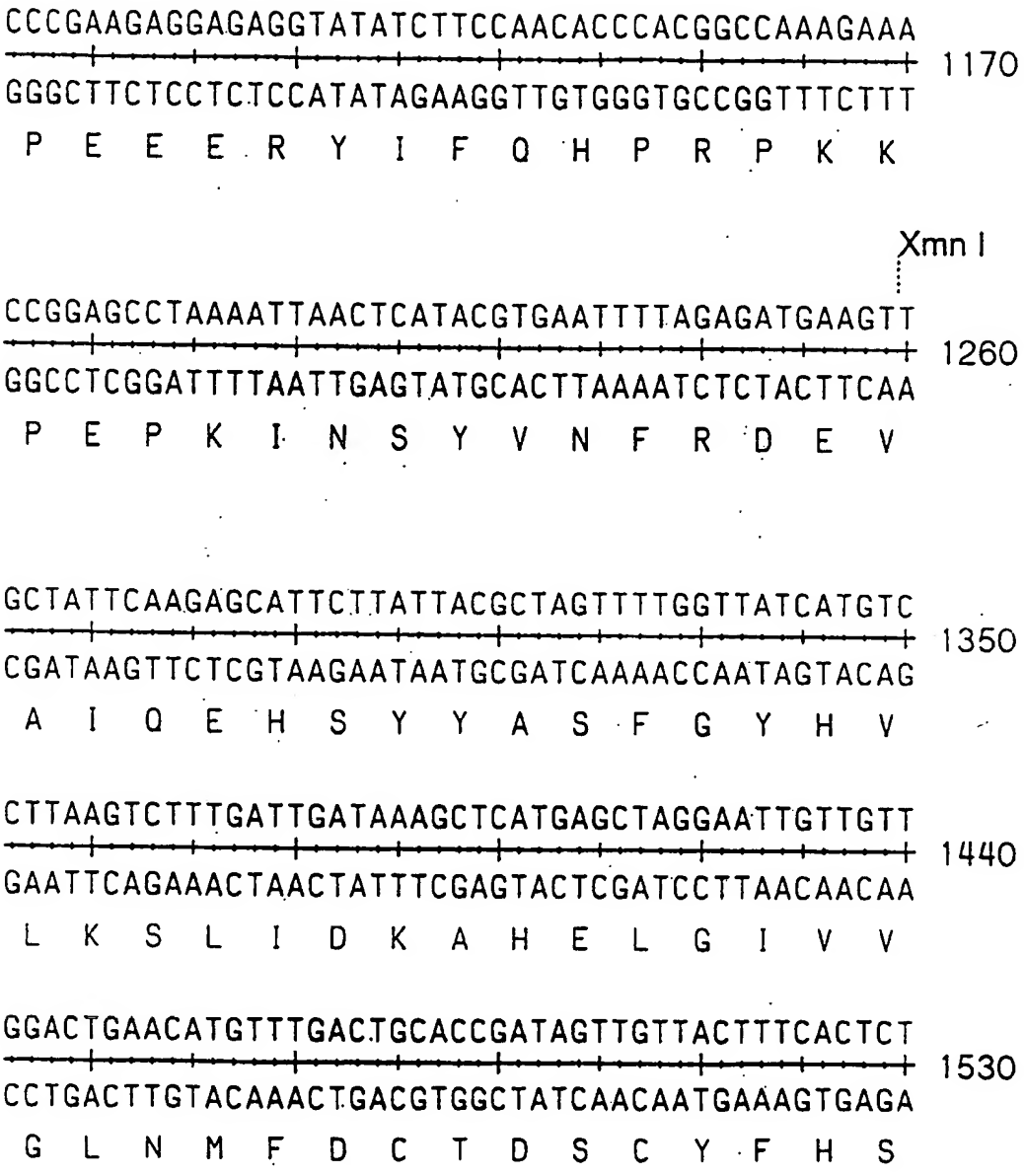


Fig.5 Sheet 6

Fig. 5 SHEET 5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Xmn I
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1260
GGCCTCGGATTTTAATTGAGTATGCACTTAAAATCTCTACTTCAA
P E P K I N S Y V N F R D E V
GCTATTCAAGAGCATTCTTATTACGCTAGTTTTGGTTATCATGTC
1350
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A I Q E H S Y Y A S F G Y H V
CTTAAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTGTTGTT
1440
GAATTCAGAAACTAACTATTTCGAGTACTCGATCCTTAACAACAA
L K S L I D K A H E L G I V V
GGACTGAACATGTTTGACTGCACCGATAGTTGTTACTTTCACTCT
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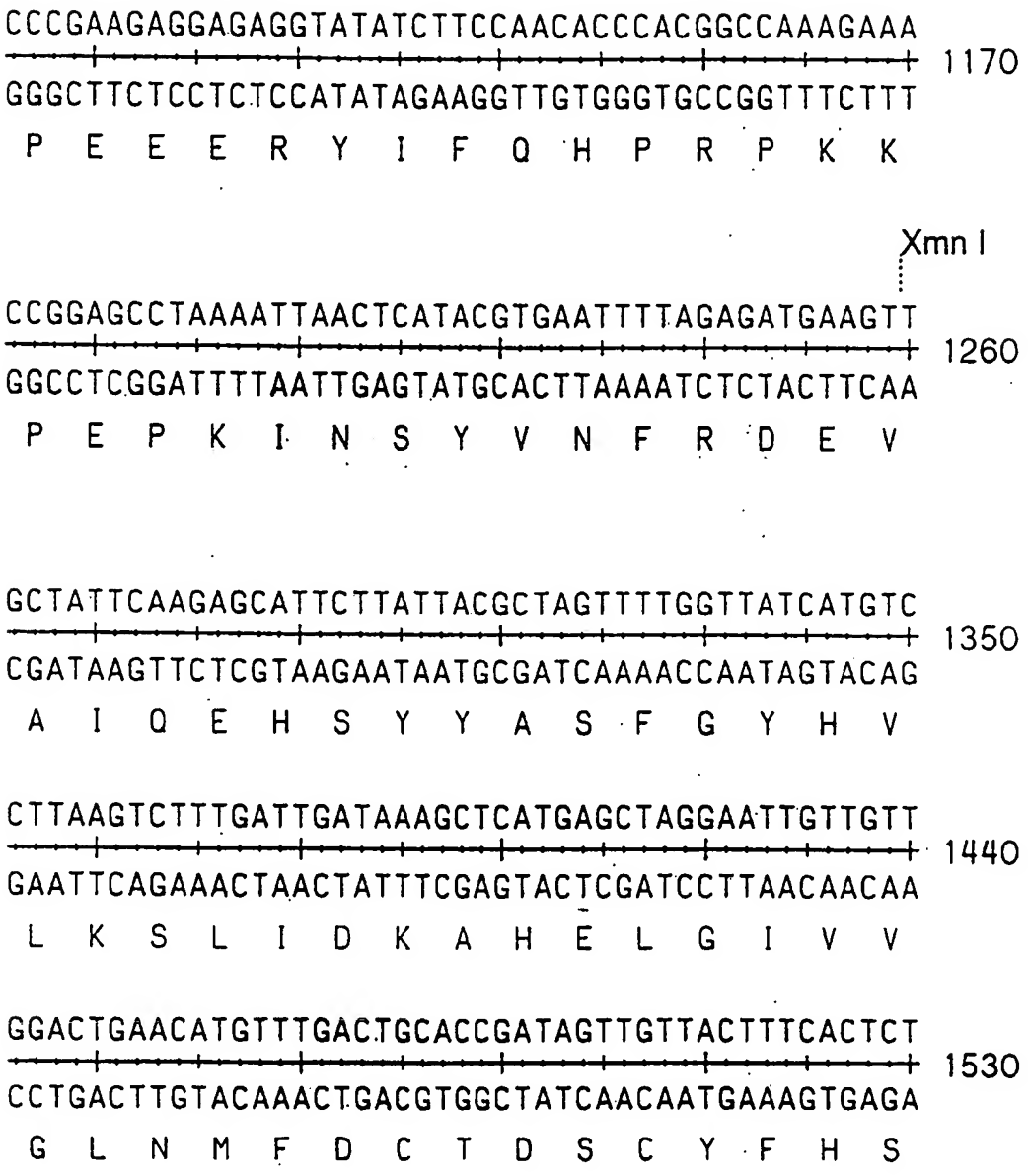
G L N M F D C T D S C Y F H S

Fig. 5 SHEET 6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P E E E R Y I F Q H P R P K K
Xmn I
CCGGAGCCTAAAATTAACTCATACGTGAATTTTAGAGATGAAGTT
1260
GGCCTCGGATTTTAATTGAGTATGCACTTAAAATCTCTACTTCAA
P E P K I N S Y V N F R D E V
GCTATTCAAGAGCATTCTTATTACGCTAGTTTTGGTTATCATGTC
1350
CGATAAGTTCTCGTAAGAATAATGCGATCAAAACCAATAGTACAG
A I Q E H S Y Y A S F G Y H V
CTTAAGTCTTTGATTGATAAAGCTCATGAGCTAGGAATTGTTGTT
1440
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L K S L I D K A H E L G I V V
GGACTGAACATGTTTGACTGCACCGATAGTTGTTACTTTCACTCT
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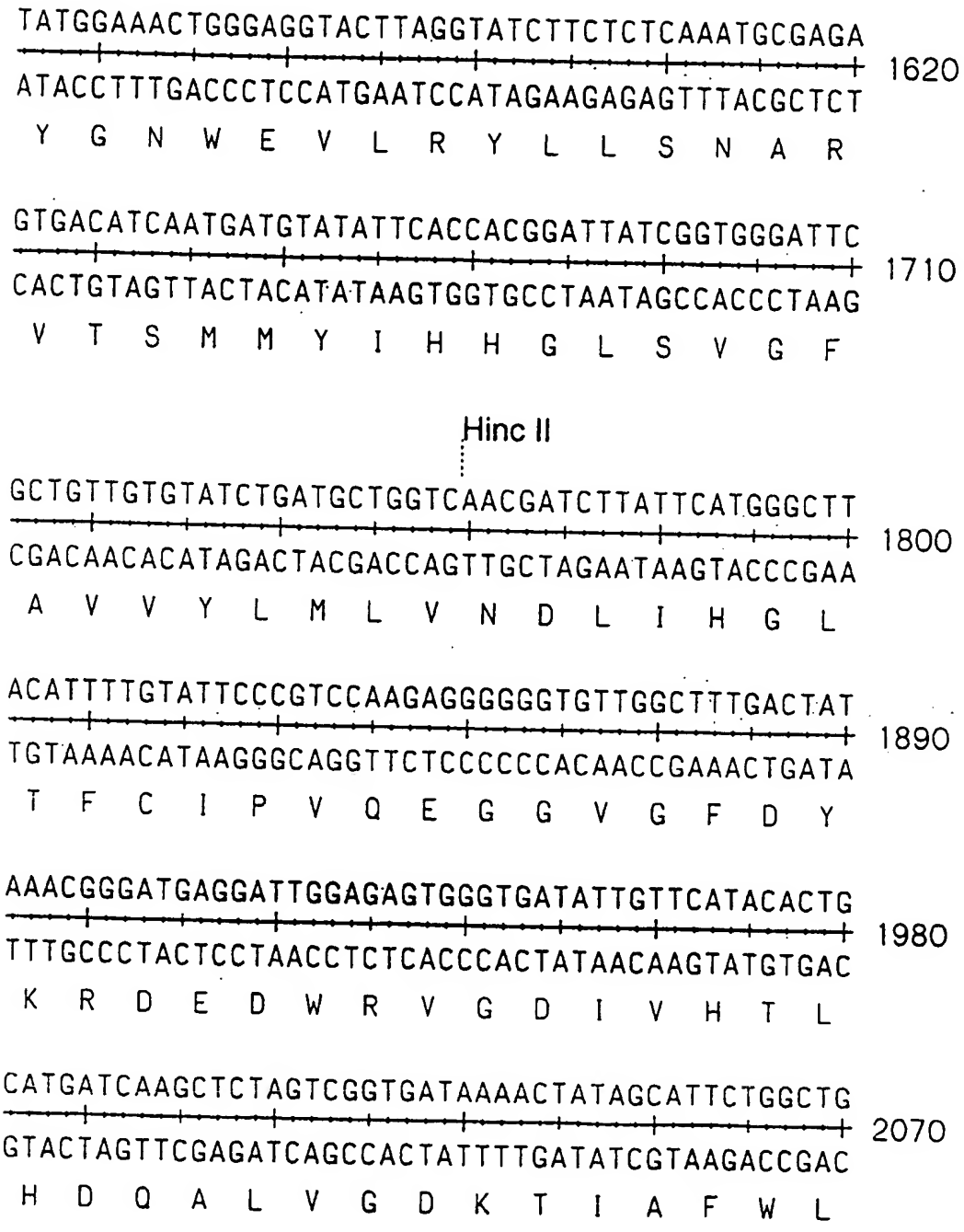
G L N M F D C T D S C Y F H S

Fig. 5 SHEET 6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GTGACATCAATGATGTATATTCACCACGGATTATCGGTGGGATTC
1710
CACTGTAGTTACTACATATAAGTGGTGCCTAATAGCCACCCTAAG
V T S M M Y I H H G L S V G F
Hinc II
GCTGTTGTGTATCTGATGCTGGTCAACGATCTTATTCATGGGCTT
1800
CGACAACACATAGACTACGACCAGTTGCTAGAATAAGTACCCGAA
A V V Y L M L V N D L I H G L
ACATTTTGTATTCCCGTCCAAGAGGGGGGTGTTGGCTTTGACTAT
1890
TGTAAAACATAAGGGCAGGTTCTCCCCCCACAACCGAAACTGATA
T F C I P V Q E G G V G F D Y
AAACGGGATGAGGATTGGAGAGTGGGTGATATTGTTCATACACTG
1980
TTTGCCCTACTCCTAACCTCTCACCCACTATAACAAGTATGTGAC
K R D E D W R V G D I V H T L
CATGATCAAGCTCTAGTCGGTGATAAAACTATAGCATTCTGGCTG
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Fig. 5 SHEET 8

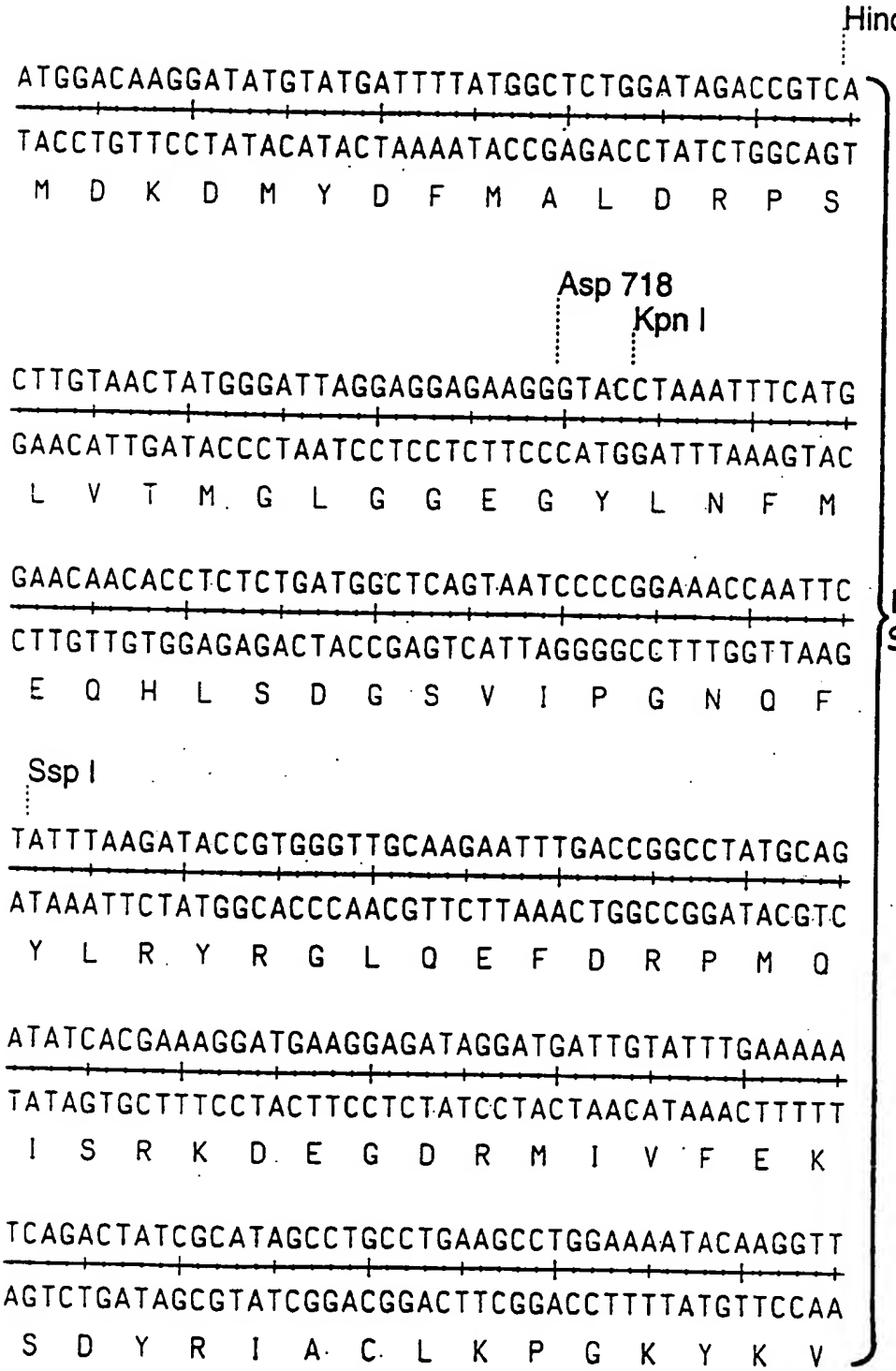

Fig.5 Sheet 10

Fig. 5 SHEET 9

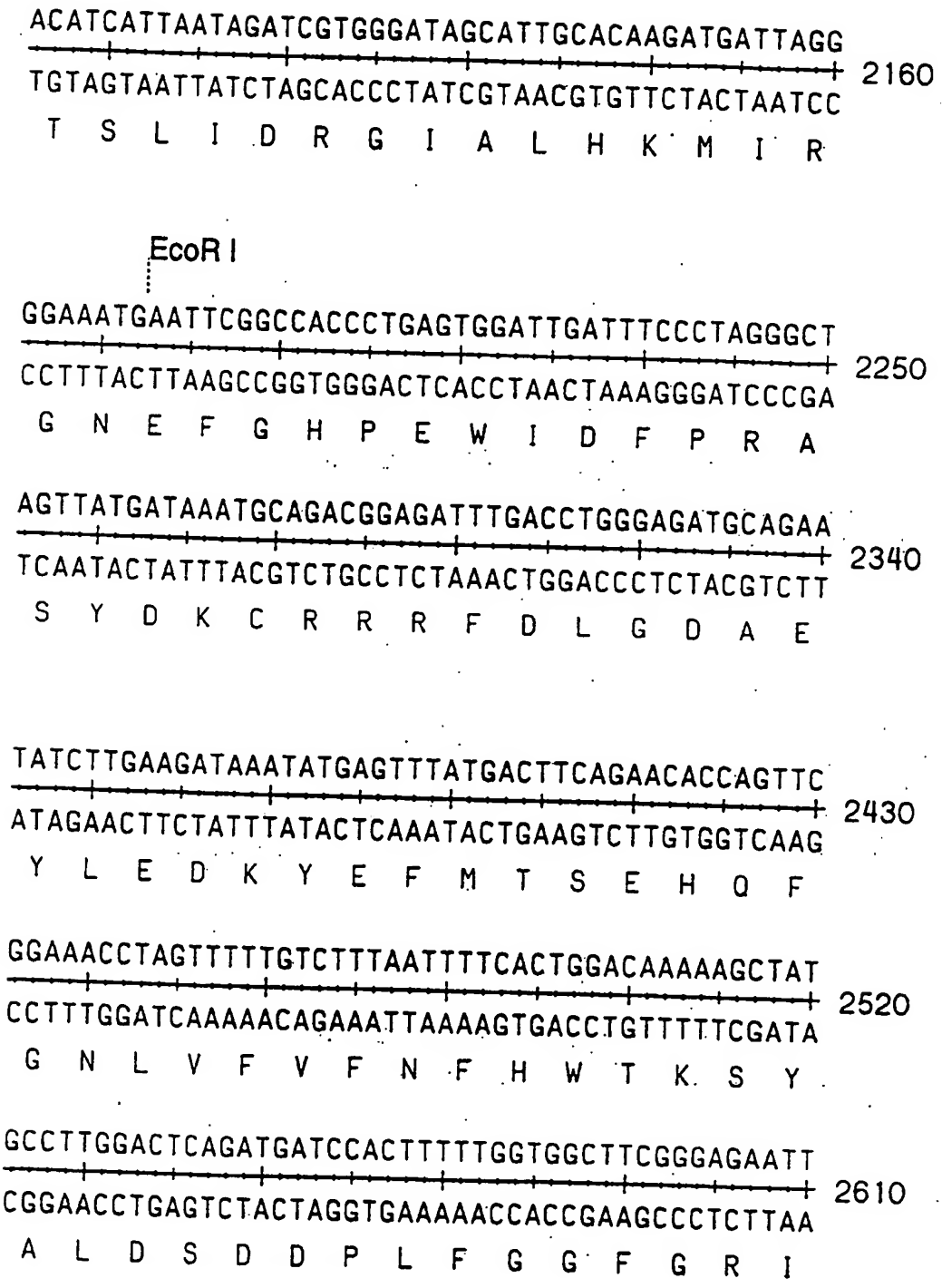

Fig. 5 SHEET 10

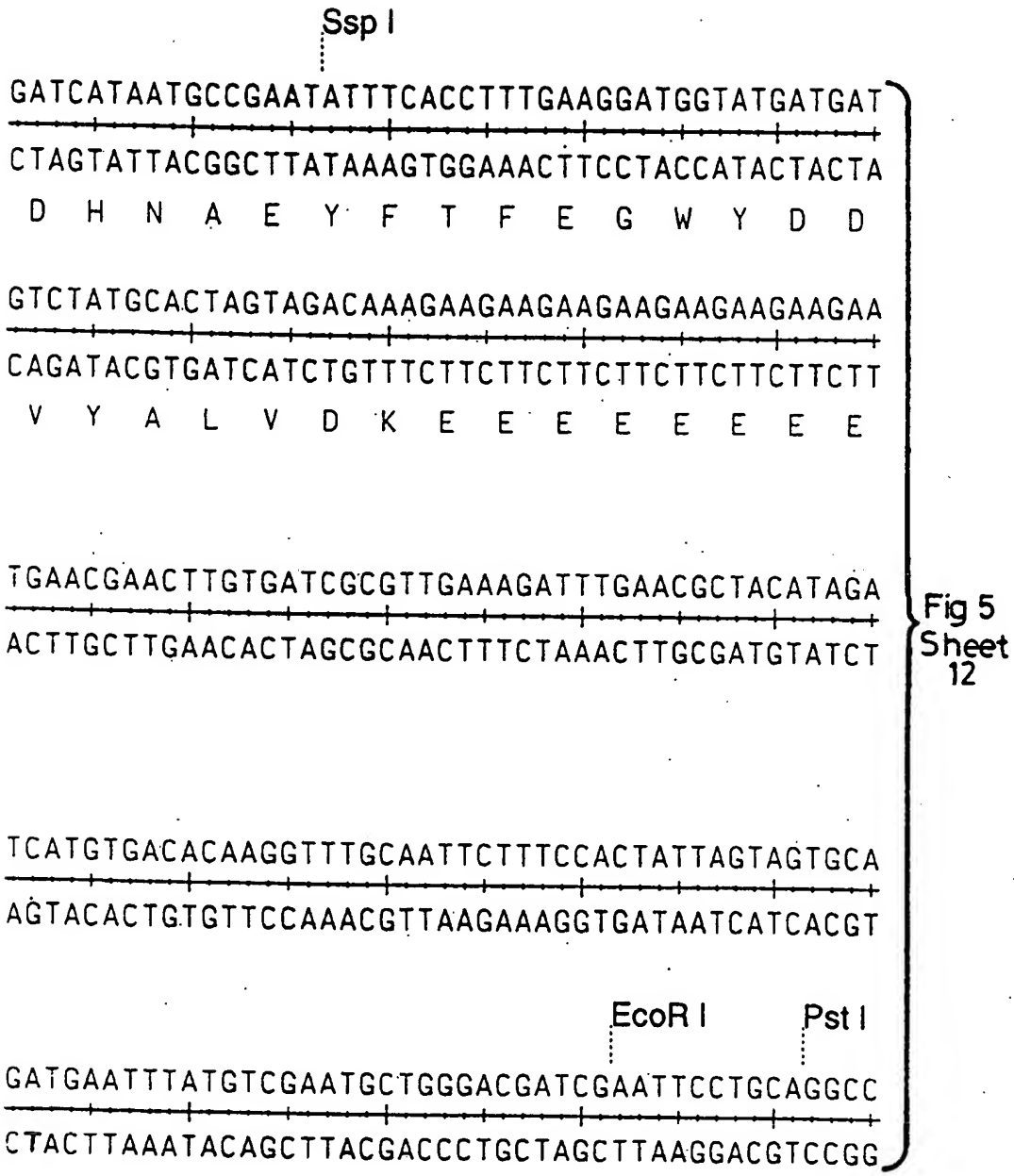

Fig 5 Sheet 12

Fig. 5 SHEET 11

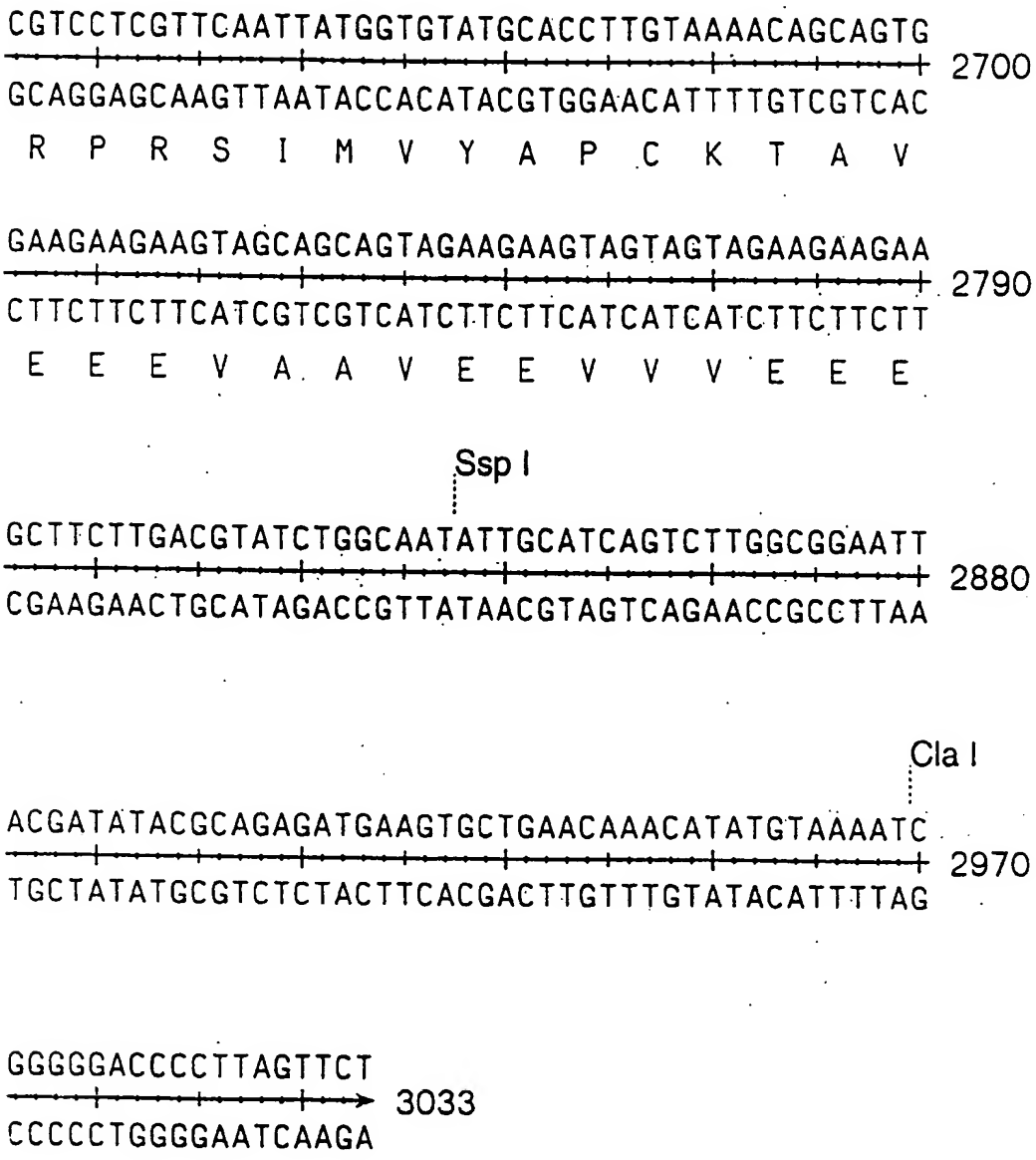

Fig. 5 SHEET 12

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LLNLDPTLEPYLDHFRHRMKRYVDQKMLIEKYEGPLEEFAQGYLKFGFNR
  100       110       120       130       140
 230       240       250       260       270
SATGITYREWALGAQSAALIGDFNNWDANADIMTRNEFGVWEIFLPNNVD
... I.YREWA :AQ.A.:IGDFN.W:::.::M.:::FGVW.I :P: VD
EDGCIVYREWAPAAQEAEVIGDFNGWNGSNHMMEKDQFGVWSIRIPD-VD
  150       160       170       180       190
 280       290       300       310       320
GSPAIPHGSRVKIRMDTPSGV-KDSIPAWINYSLQLPDEI--PYNGIHYD
:.P.IPH.SRVK:R.. :GV D.IPAWI:Y: . :.: PY:G:.D
SKPVIPHNSRVKFRFKHGNGVWVDRIPAWIKYATADATKFAAPYDGVYWD
    200       210       220       230       240
    330       340       350       360       370
PPEEERYIFQHPRPKKPKSLRIYESHIGMSSPEPKINSYVNFRDEVLPRI
PP .ERY F:.PRP KP:: RIYE:H:GMSS:EP::NSY :F D:VLPRI
PPPSERYHFKYPRPPKPRAPRIYEAHVGMSSSEPRVNSYREFADDVLPRI
    250       260       270       280       290
    380       390       400       410       420
KKLGYNALQIMAIQEHSYYASFGYHVTNFFAPSSRFGTPDDLKSLIDKAH
K .YN::Q:MAI EHSYY:SFGYHVTNFFA S:R:G.P:DLK LIDKAH
KANNYNTVQLMAIMEHSYYGSFGYHVTNFFAVSNRYGNPEDLKYLIDKAH
    300       310       320       330       340
    430       440       450       460       470
ELGIVVLMDIVHSHASNNTLDGLNMFDC---TDSCYFHSGARGYHWMWDS
.LG: VL:D:VHSHASNN. DGLN FD :::.YFH:G.RGYH :WDS
SLGLQVLVDVVHSHASNNVTDGLNGFDIGQGSQESYFHAGERGYHKLWDS
    350       360       370       380       390
       480       490       500       510       520
RLFNYGNWEVLRYLLSNARWWLDAFKFDGFRFDGVTSMMYIHHGLSVGFT
RLFNY:NWEVLR:LLSN RWWL:.::FDGFRFDG:TSM:Y:HHG::GFT
RLFNYANWEVLRFLLSNLRWWLEEYNFDGFRFDGITSMLYVHHGINMGFT
    400       410       420       430       440
       530       540       550       560       570
GNYEEYFGLATDVDAVVYLMLVNDLIHGLFPDAITIGEDVSGMPTFCIPV
GNY:EYF: ATDVDAVVYLML.N:LIH :FPDA..I:EDVSGMP.:. PV
GNYNEYFSEATDVDAVVYLMLANNLIHKIFPDATVIAEDVSGMPGLSRPV
    450       460       470       480       490
       580       590       600       610       620
QEGGVGFDYRLHMAIADKRIELLK-KRDEDWRVGDIVHTLTNRRWSEKCV
 EGG:GFDYRL MAI:DK:I: LK K.DEDW.: ::. :LTNRR.:EKC:
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    500       510       520       530       540
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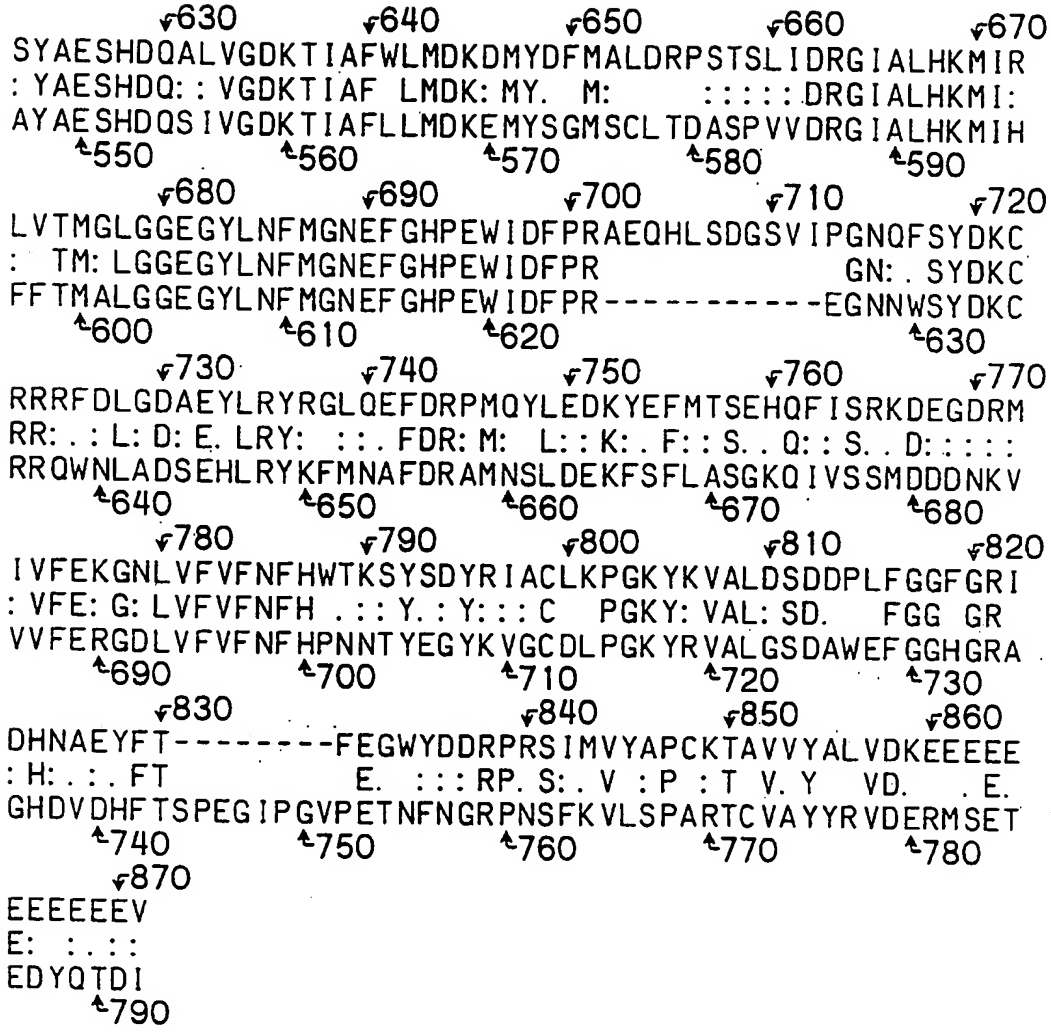
Fig. 6 SHEET 1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: YAESHDQ: : VGDKTIAF LMDK: MY. M: : : : : DRGIALHKMI:
AYAESHDQSIVGDKTIAFLLMDKEMYSGMSCLTDASPVVDRGIALHKMIH
550 560 570 580 590
680 690 700 710 720
LVTMGLGGEGYLNFMGNEFGHPEWIDFPRAEQHLSDGSVIPGNQFSYDKC
: TM: LGGEGYLNFMGNEFGHPEWIDFPR GN: . SYDKC
FFTMALGGEGYLNFMGNEFGHPEWIDFPR----------EGNNWSYDKC
600 610 620 630
730 740 750 760 770
RRRFDLGDAEYLRYRGLQEFDRPMQYLEDKYEFMTSEHQFISRKDEGDRM
RR: . : L: D: E. LRY: : : . FDR: M: L: : K: . F: : S. . Q: : S. . D: : : :
RRQWNLADSEHLRYKFMNAFDRAMNSLDEKFSFLASGKQIVSSMDDDNKV
640 650 660 670 680
780 790 800 810 820
IVFEKGNLVFVFNFHWTKSYSDYRIACLKPGKYKVALDSDDPLFGGFGRI
: VFE: G: LVFVFNFH . : : Y. : Y: : : C PGKY: VAL: SD. FGG GR
VVFERGDLVFVFNFHPNNTYEGYKVGCDLPGKYRVALGSDAWEFGGHGRA
690 700 710 720 730
830 840 850 860
DHNAEYFT--------FEGWYDDRPRSIMVYAPCKTAVVYALVDKEEEEE
: H: . : . FT E. : : : RP. S: . V : P : T V. Y VD. . E.
GHDVDHFTSPEGIPGVPETNFNGRPNSFKVLSPARTCVAYYRVDERMSET
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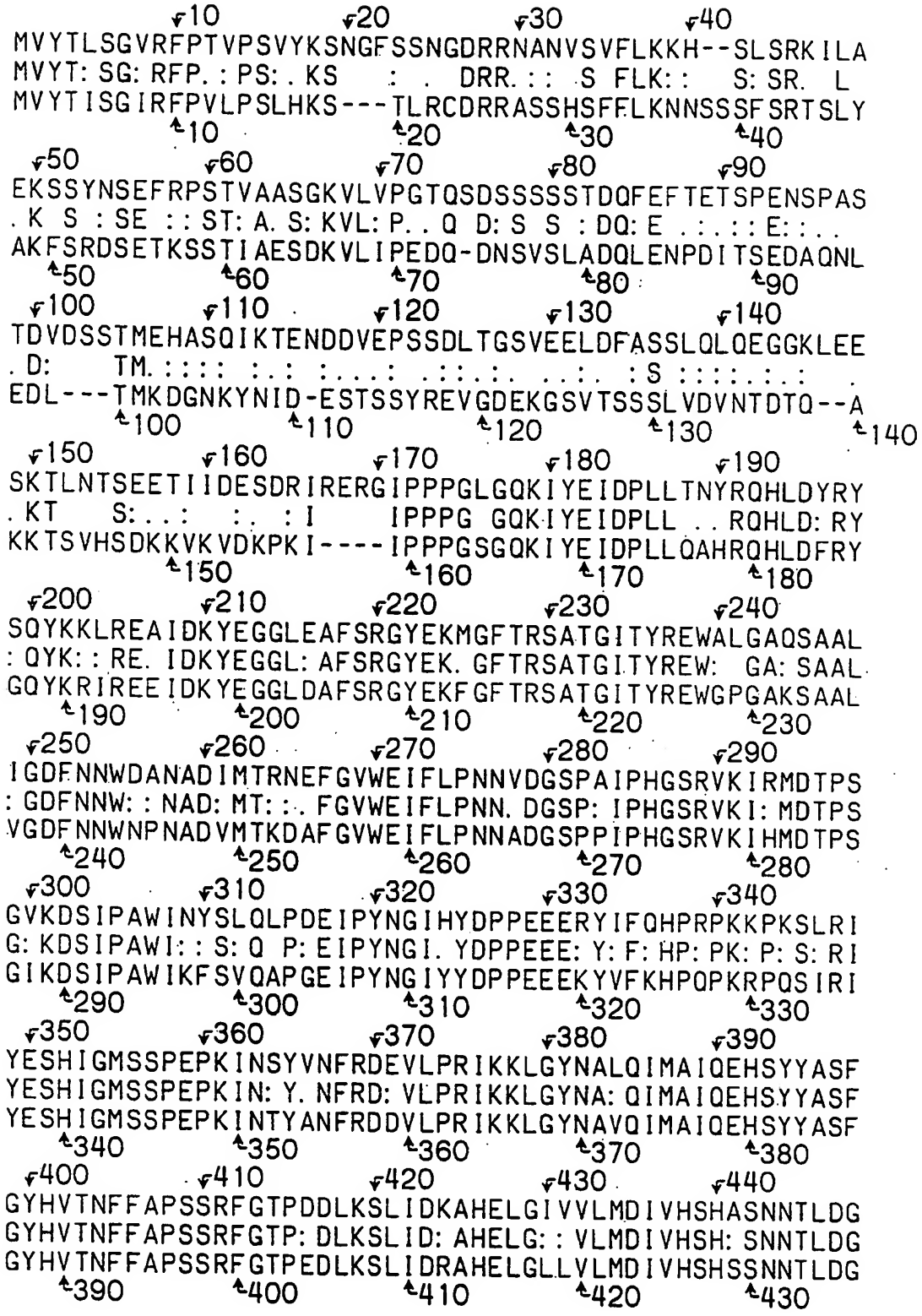
870
EEEEEEV
E: : . : :
EDYQTDI
790

Fig. 6 SHEET 2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MVYTISGIRFPVLPSLHKS---TLRCDRRASSHSFFLKNNSSSFSRTSLY
10 20 30 40
50 60 70 80 90
EKSSYNSEFRPSTVAASGKVLVPGTQSDSSSSSTDQFEFTETSPENSPAS
. K S : SE : : ST: A. S: KVL: P. . Q D: S S : DQ: E . : . : : E: : . .
AKFSRDSETKSSTIAESDKVLIPEDQ-DNSVSLADQLENPDITSEDAQNL
50 60 70 80 90
100 110 120 130 140
TDVDSSTMEHASQIKTENDDVEPSSDLTGSVEELDFASSLQLQEGGKLEE
. D: TM. : : : : : . : : . . . : . : : . : . . . : . : S : : : : . : : .
EDL---TMKDGNKYNID-ESTSSYREVGDEKGSVTSSSLVDVNTDTQ--A
100 110 120 130 140
150 160 170 180 190
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. KT S: . . : : . : I IPPPG GQKIYEIDPLL . . RQHLD: RY
KKTSVHSDKKVKVDKPKI----IPPPGSGQKIYEIDPLLQAHRQHLDFRY
150 160 170 180
200 210 220 230 240
SQYKKLREAIDKYEGGLEAFSRGYEKMGFTRSATGITYREWALGAQSAAL
: QYK: : RE. IDKYEGGL: AFSRGYEK. GFTRSATGITYREW: GA: SAAL
GQYKRIREEIDKYEGGLDAFSRGYEKFGFTRSATGITYREWGPGAKSAAL
190 200 210 220 230
250 260 270 280 290
IGDFNNWDANADIMTRNEFGVWEIFLPNNVDGSPAIPHGSRVKIRMDTPS
: GDFNNW: : NAD: MT: : . FGVWEIFLPNN. DGSP: IPHGSRVKI: MDTPS
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240 250 260 270 280
300 310 320 330 340
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G: KDSIPAWI: : S: Q P: EIPYNGI. YDPPEEE: Y: F: HP: PK: P: S: RI
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290 300 310 320 330
350 360 370 380 390
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YESHIGMSSPEPKINTYANFRDDVLPRIKKLGYNAVQIMAIQEHSYYASF
340 350 360 370 380
400 410 420 430 440
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390 400 410 420 430

Fig. 7 SHEET 1

```
 ↓450       ↓460       ↓470       ↓480       ↓490
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LNMFD TD:  YFH: G: RGYHWMWDSRLFNYG: WEVLRYLLSNARWWLD. :
LNMFDGTDGHYFHPGSRGYHWMWDSRLFNYGSWEVLRYLLSNARWWLDEY
   ↑440       ↑450       ↑460       ↑470       ↑480
 ↓500       ↓510       ↓520       ↓530       ↓540
KFDGFRFDGVTSMMYIHHGLSVGFTGNYEEYFGLATDVDAVVYLMLVNDL
KFDGFRFDGVTSMMY. HHGL  V: FTGNY. EYFGLATDV: AVVY: MLVNDL
KFDGFRFDGVTSMMYTHHGLQVSFTGNYSEYFGLATDVEAVVYMMLVNDL
   ↑490       ↑500       ↑510       ↑520       ↑530
 ↓550       ↓560       ↓570       ↓580       ↓590
IHGLFPDAITIGEDVSGMPTFCIPVQEGGVGFDYRLHMAIADKRIELLKK
IHGLFP: A: : IGEDVSGMPTFC: P. Q: GG: GF: YRLHMA: ADK: IELLKK
IHGLFPEAVSIGEDVSGMPTFCLPTQDGGIGFNYRLHMAVADKWIELLKK
   ↑540       ↑550       ↑560       ↑570       ↑580
 ↓600       ↓610       ↓620       ↓630       ↓640
RDEDWRVGDIVHTLTNRRWSEKCVSYAESHDQALVGDKTIAFWLMDKDMY
: DEDWR: GDIVHTLTNRRW EKCV YAESHDQALVGDKT: AFWLMDKDMY
QDEDWRMGDIVHTLTNRRWLEKCVVYAESHDQALVGDKTLAFWLMDKDMY
   ↑590       ↑600       ↑610       ↑620       ↑630
 ↓650       ↓660       ↓670       ↓680       ↓690
DFMALDRPSTSLIDRGIALHKMIRLVTMGLGGEGYLNFMGNEFGHPEWID
DFMALDRPST: LIDRGIALHKMIRL: TMGLGGEGYLNFMGNEFGHPEWID
DFMALDRPSTPLIDRGIALHKMIRLITMGLGGEGYLNFMGNEFGHPEWID
   ↑640       ↑650       ↑660       ↑670       ↑680
 ↓700       ↓710       ↓720       ↓730       ↓740
FPRAEQHLSDGSVIPGNQFSYDKCRRRFDLGDAEYLRYRGLQEFDRPMQY
FPR: EQHL: : G. : : PGN:  SYDKCRRRFDLGDA: YLRY: G: QEFDR: MQ.
FPRGEQHLPNGKIVPGNNNSYDKCRRRFDLGDADYLRYHGMQEFDRAMQH
   ↑690       ↑700       ↑710       ↑720       ↑730
 ↓750       ↓760       ↓770       ↓780       ↓790
LEDKYEFMTSEHQFISRKDEGDRMIVFEKGNLVFVFNFHWTKSYSDYRIA
LE:. Y. FMTSEHQ: ISRK: EGDR: I: FE: : NLVFVFNFHWT: SYSDY: : :
LEETYGFMTSEHQYISRKNEGDRVIIFERDNLVFVFNFHWTNSYSDYKVG
   ↑740       ↑750       ↑760       ↑770       ↑780
 ↓800       ↓810       ↓820       ↓830       ↓840
CLKPGKYKVALDSDDPLFGGFGRIDHNAEYFTFEGWYDDRPRSIMVYAPC
CLKPGKYK:. LDSDD. LFGGF. R: : H. AEYFT EGWYDDRPRS: : VYAP.
CLKPGKYKIVLDSDDTLFGGFNRLNHTAEYFTSEGWYDDRPRSFLVYAPS
   ↑790       ↑800       ↑810       ↑820       ↑830
 ↓850       ↓860       ↓870
KTAVVYALVDKEEEEEEEEEEEVAA
: TAVVYAL. D  E. E  E . : . V. :
RTAVVYALADGVESEPIELSDGVES
   ↑840       ↑850       ↑860
```

Fig. 7 SHEET 2

```
1    --------------------TTG--AT----------
1    --------------------TTGA----------
1    --------------------GA----------
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73   TTTCTCTTAATTCCAACCAAGG-AATGAATAAAAGGAT-A
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431  CAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGC
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429  CAGCATCAACTGATGTAGATAGTTCAACAATGGAACACGC
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789  AAGCTTTTTTCTCGTGGTTATGAAACAATGGGTTTCACTCG
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Fig.8 Sheet 2

Fig. 8 SHEET 1

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Fig. 8 Sheet 3

Fig. 8 SHEET 2

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Fig. 8
SHEET 3

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1630 GATGCGTTCAAATTTGATGGATTTAGATTTGATGGTGTGAC
1629 GATGAGTTCAAATTTGATGGATTTAGATTTGATGGTGTGAC
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Fig. 8
Sheet 5

Fig. 8
SHEET 4

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Fig. 8
Sheet 6

Fig. 8
SHEET 5

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Fig. 8
SHEET 6

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Fig.8
Sheet 8

Fig.8
SHEET 7

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Fig.8 Sheet 9

Fig. 8 SHEET 8

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Fig. 8
SHEET 9

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Fig. 8 Sheet 11

Fig. 8 SHEET 10

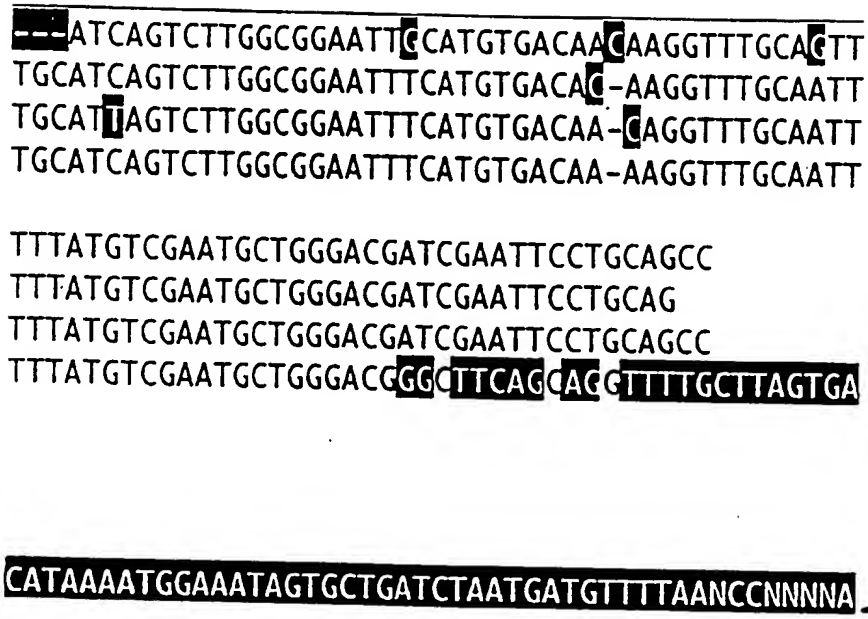

Fig. 8 Sheet 12

Fig. 8 SHEET 11

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Fig. 8 SHEET 12

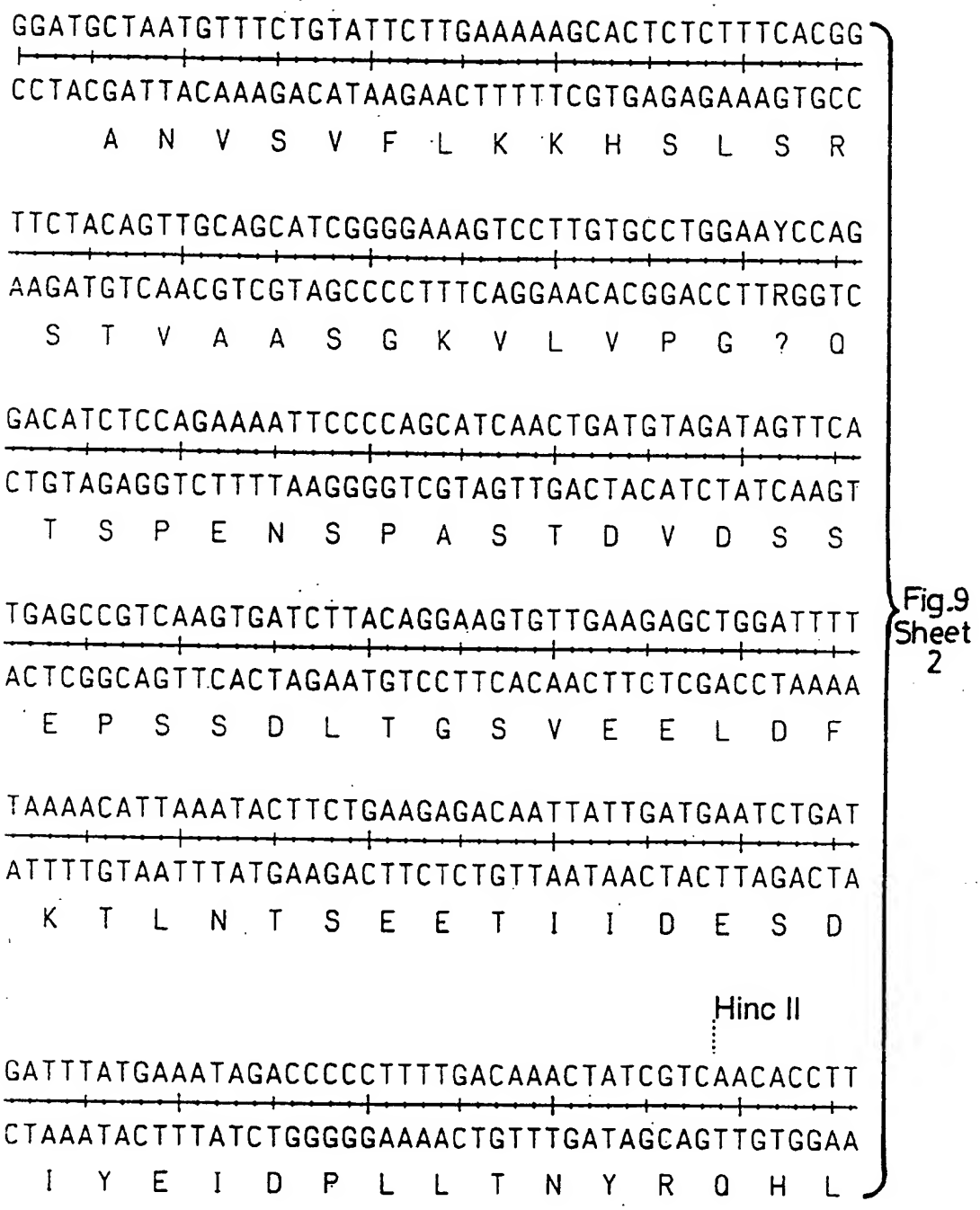

Fig.9 Sheet 2

Fig. 9 SHEET 1

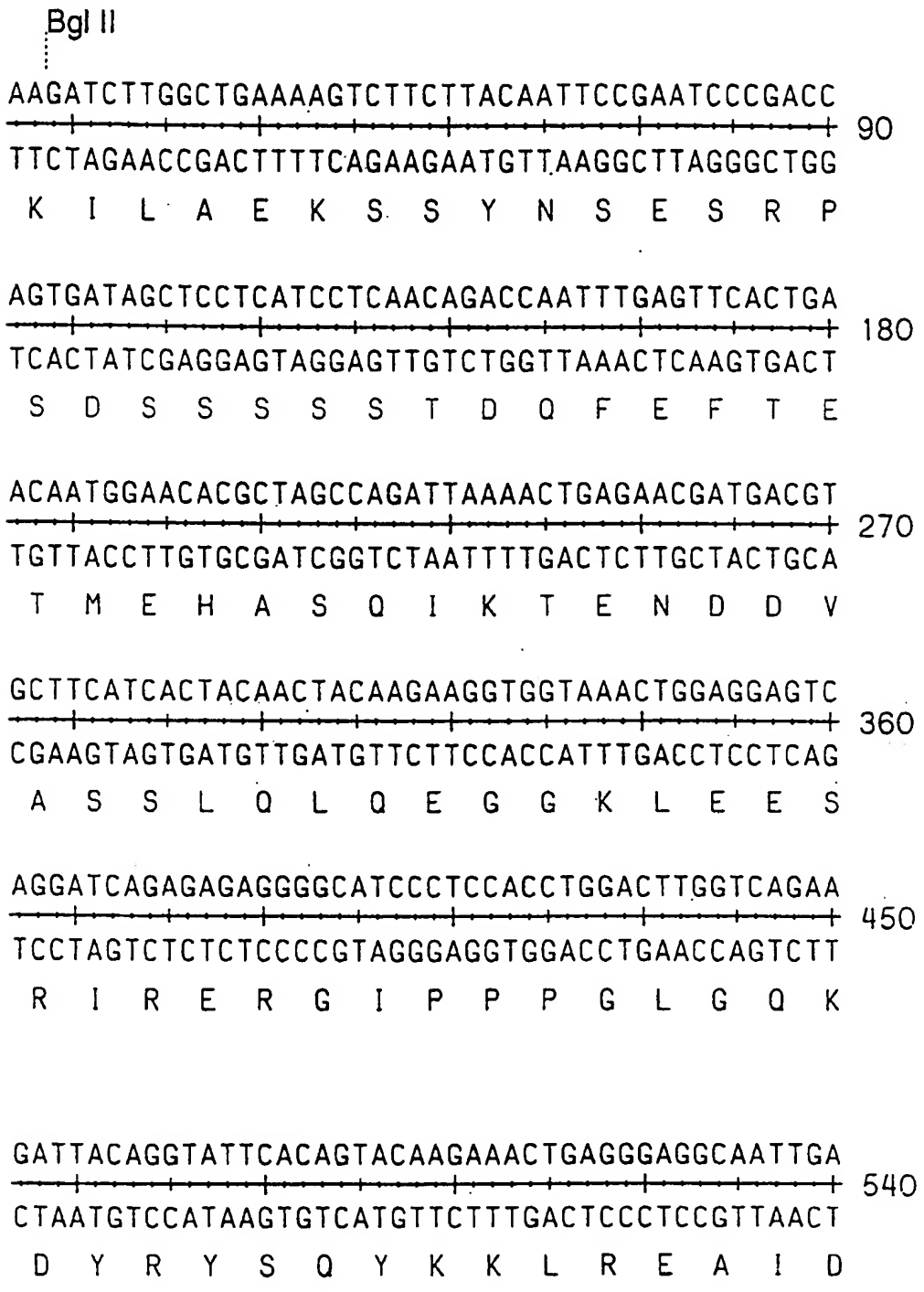
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Fig. 9 SHEET 2

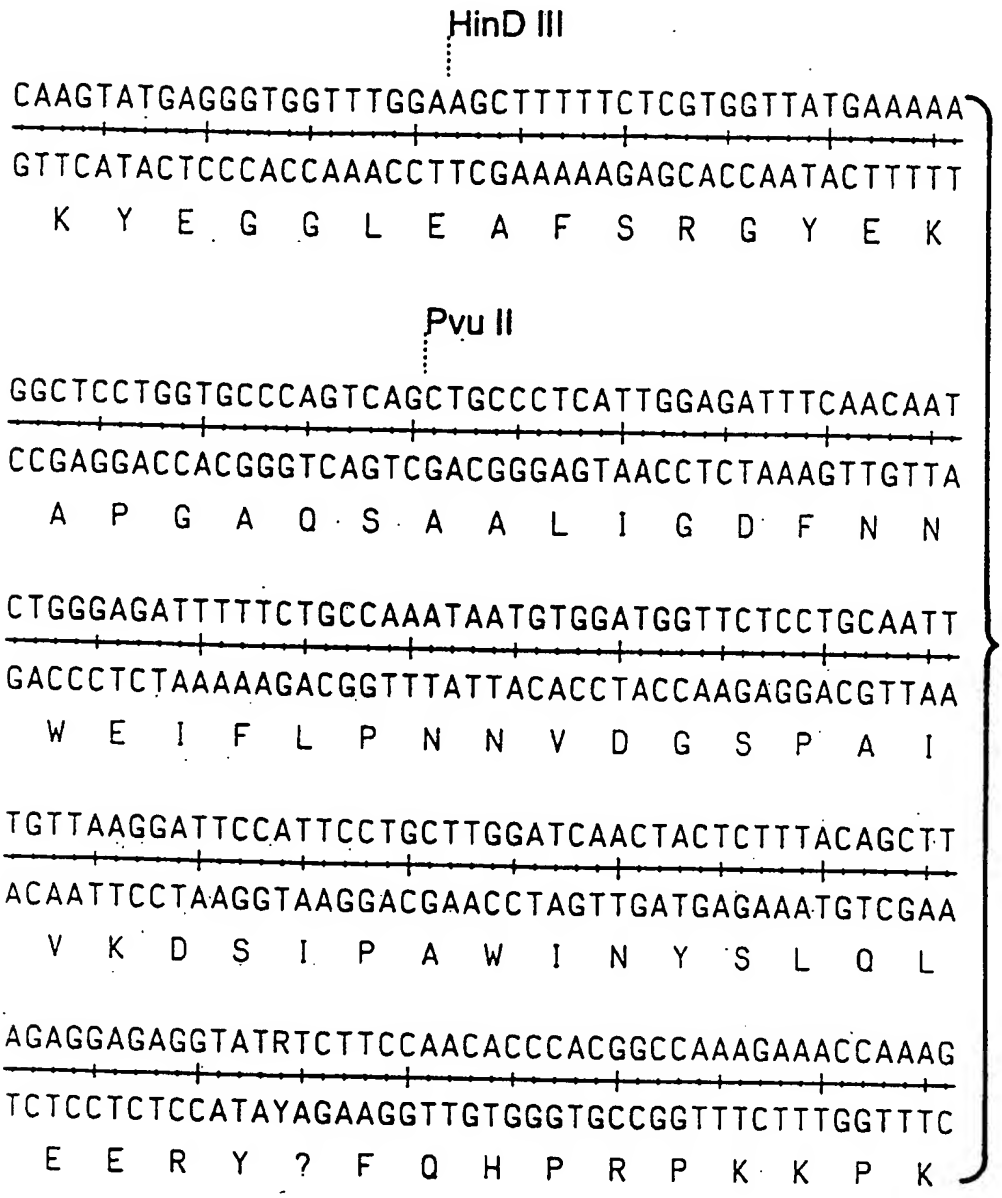
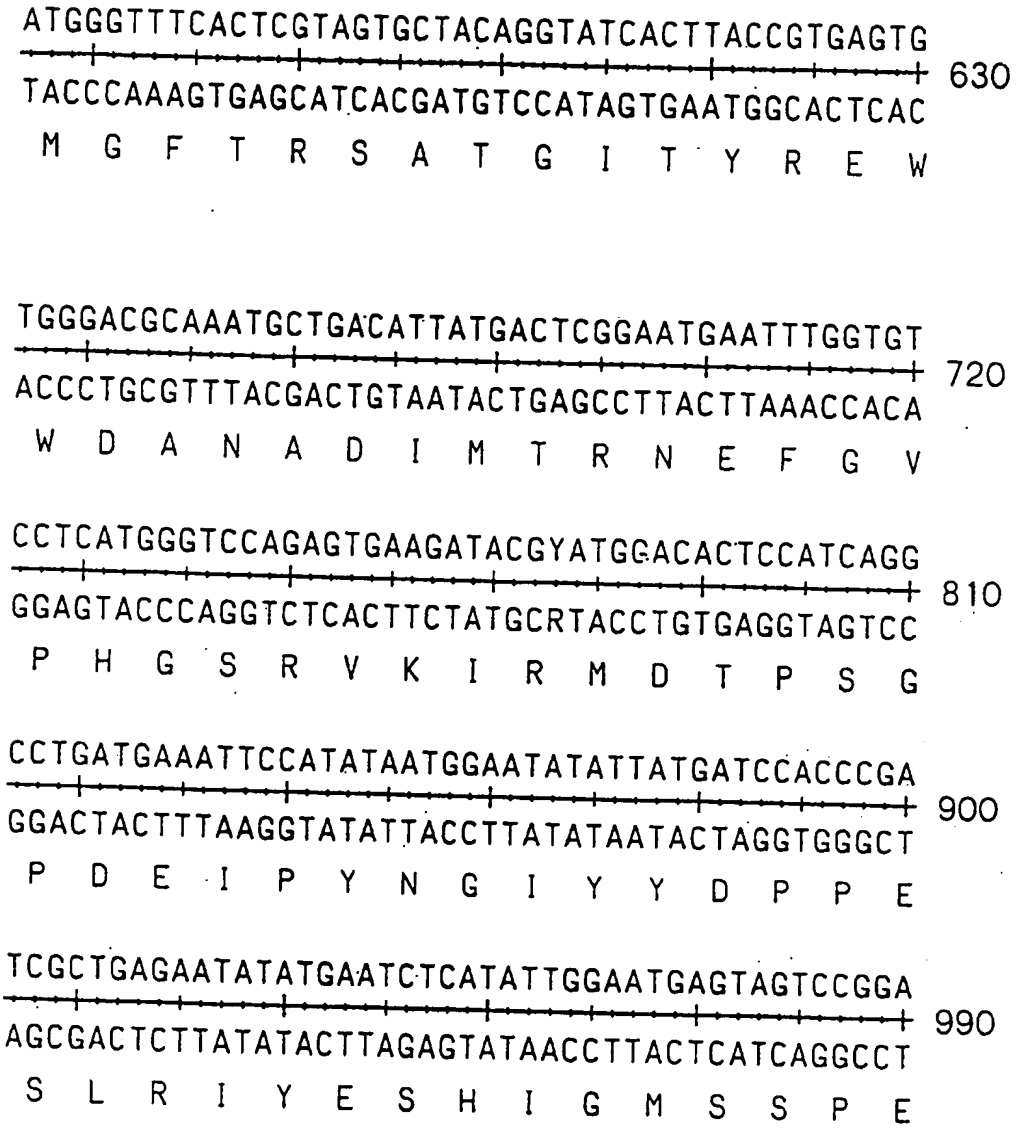


Fig.9 Sheet 4

Fig. 9 SHEET 3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720
W D A N A D I M T R N E F G V
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810
P H G S R V K I R M D T P S G
CCTGATGAAATTCCATATAATGGAATATATTATGATCCACCCGA
GGACTACTTTAAGGTATATTACCTTATATAATACTAGGTGGGCT
900
P D E I P Y N G I Y Y D P P E
TCGCTGAGAATATATGAATCTCATATTGGAATGAGTAGTCCGGA
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990
S L R I Y E S H I G M S S P E

Fig. 9 SHEET 4

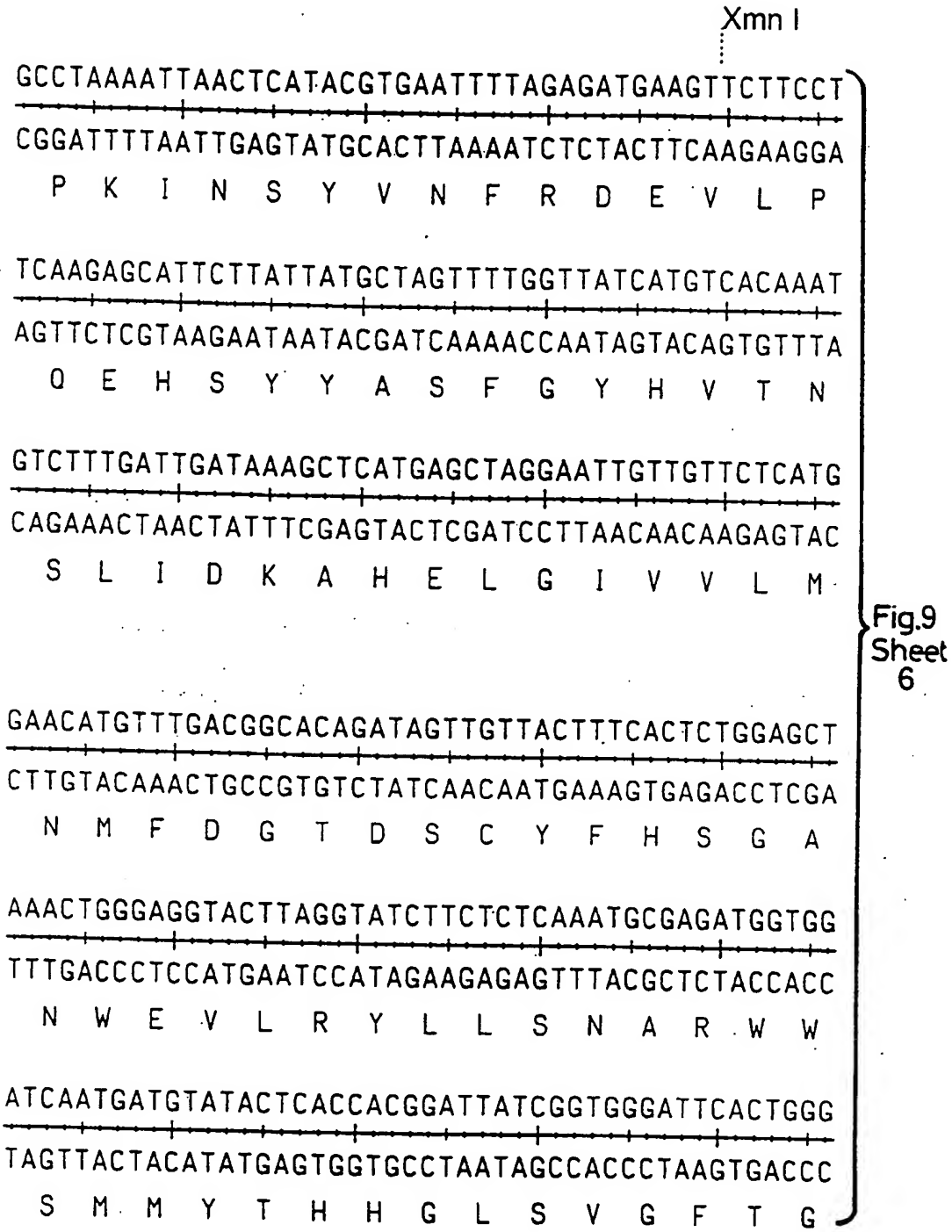
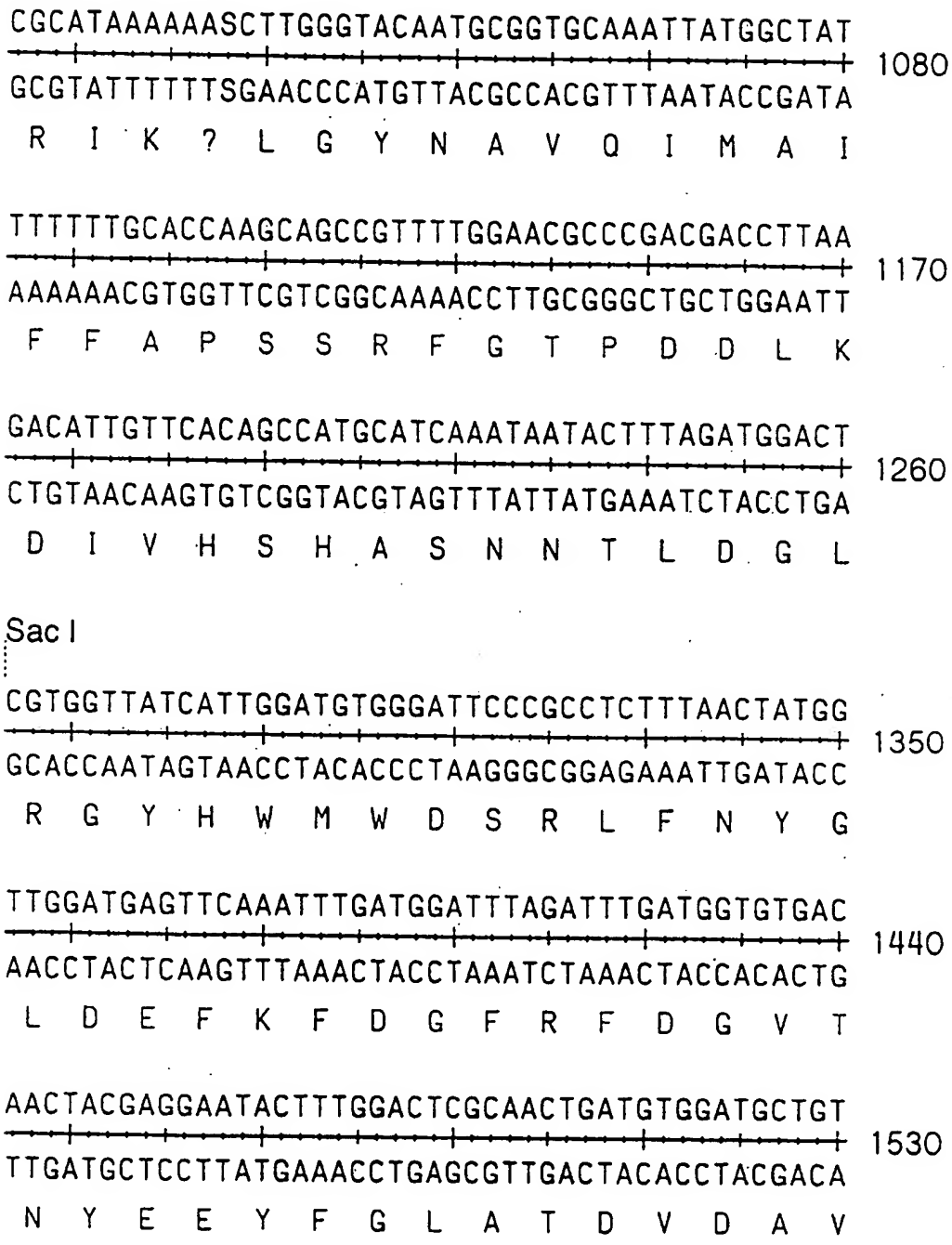


Fig.9 Sheet 6

Fig. 9 SHEET 5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1170
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F F A P S S R F G T P D D L K
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1260
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D I V H S H A S N N T L D G L
Sac I
CGTGGTTATCATTGGATGTGGGATTCCCGCCTCTTTAACTATGG
1350
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R G Y H W M W D S R L F N Y G
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1440
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L D E F K F D G F R F D G V T
AACTACGAGGAATACTTTGGACTCGCAACTGATGTGGATGCTGT
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Fig. 9 SHEET 6

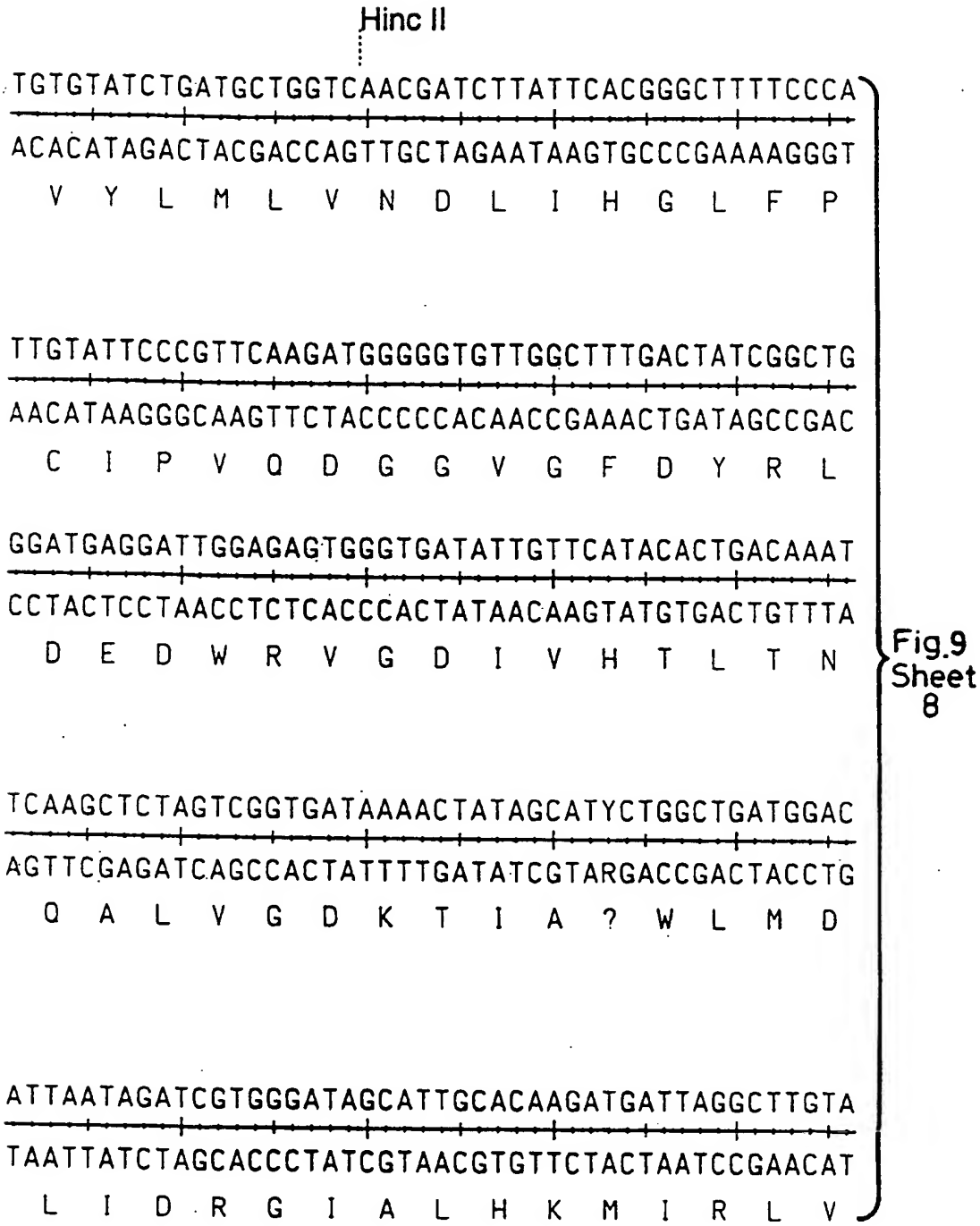
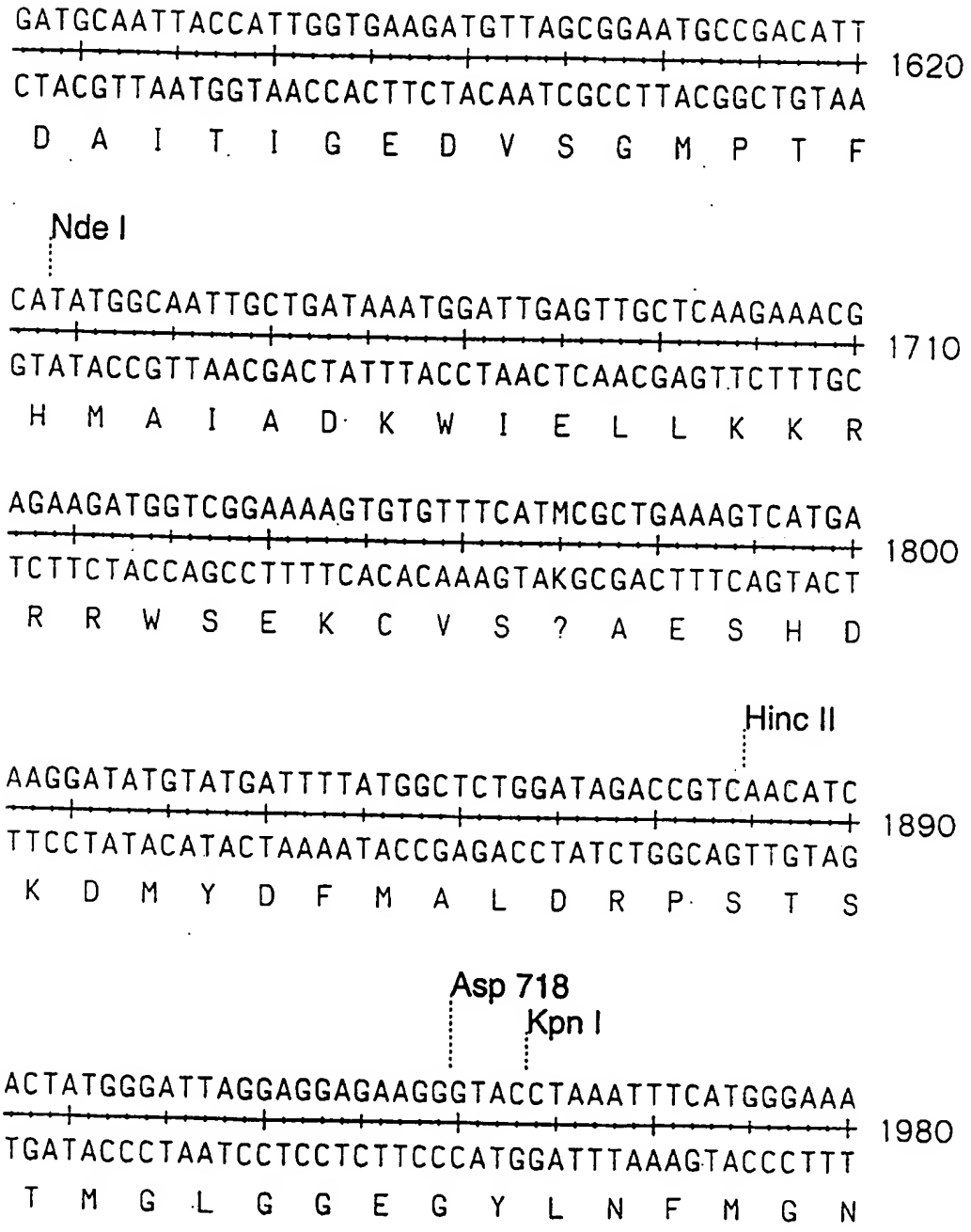


Fig.9 Sheet 8

Fig. 9 SHEET 7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Nde I
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1710
H M A I A D K W I E L L K K R
AGAAGATGGTCGGAAAAGTGTGTTTCATMCGCTGAAAGTCATGA
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1800
R R W S E K C V S ? A E S H D
Hinc II
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1890
K D M Y D F M A L D R P S T S
Asp 718
Kpn I
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1980
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Fig. 9 SHEET 8

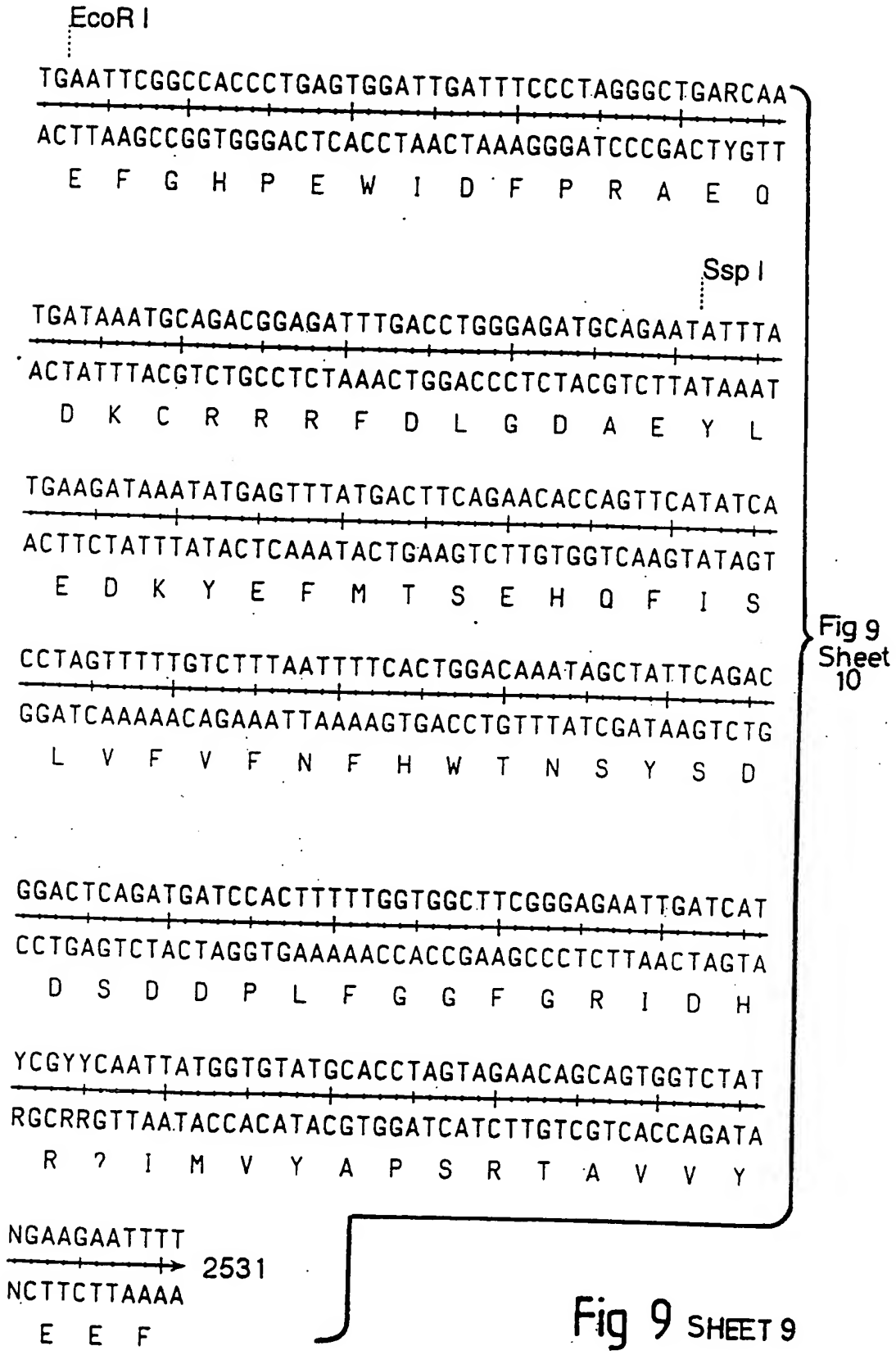

Fig 9 Sheet 10

Fig 9 SHEET 9

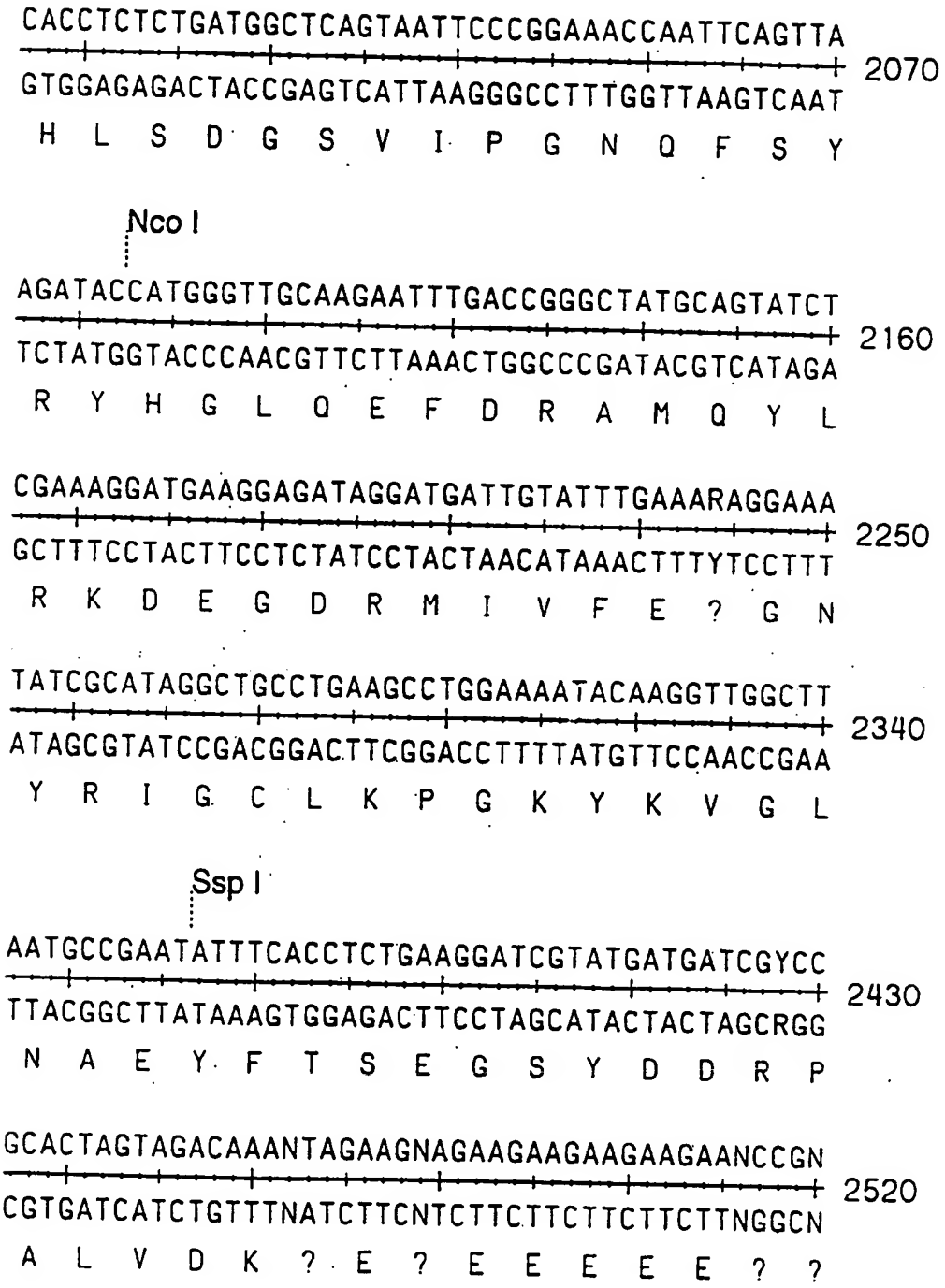

Fig. 9 SHEET 10

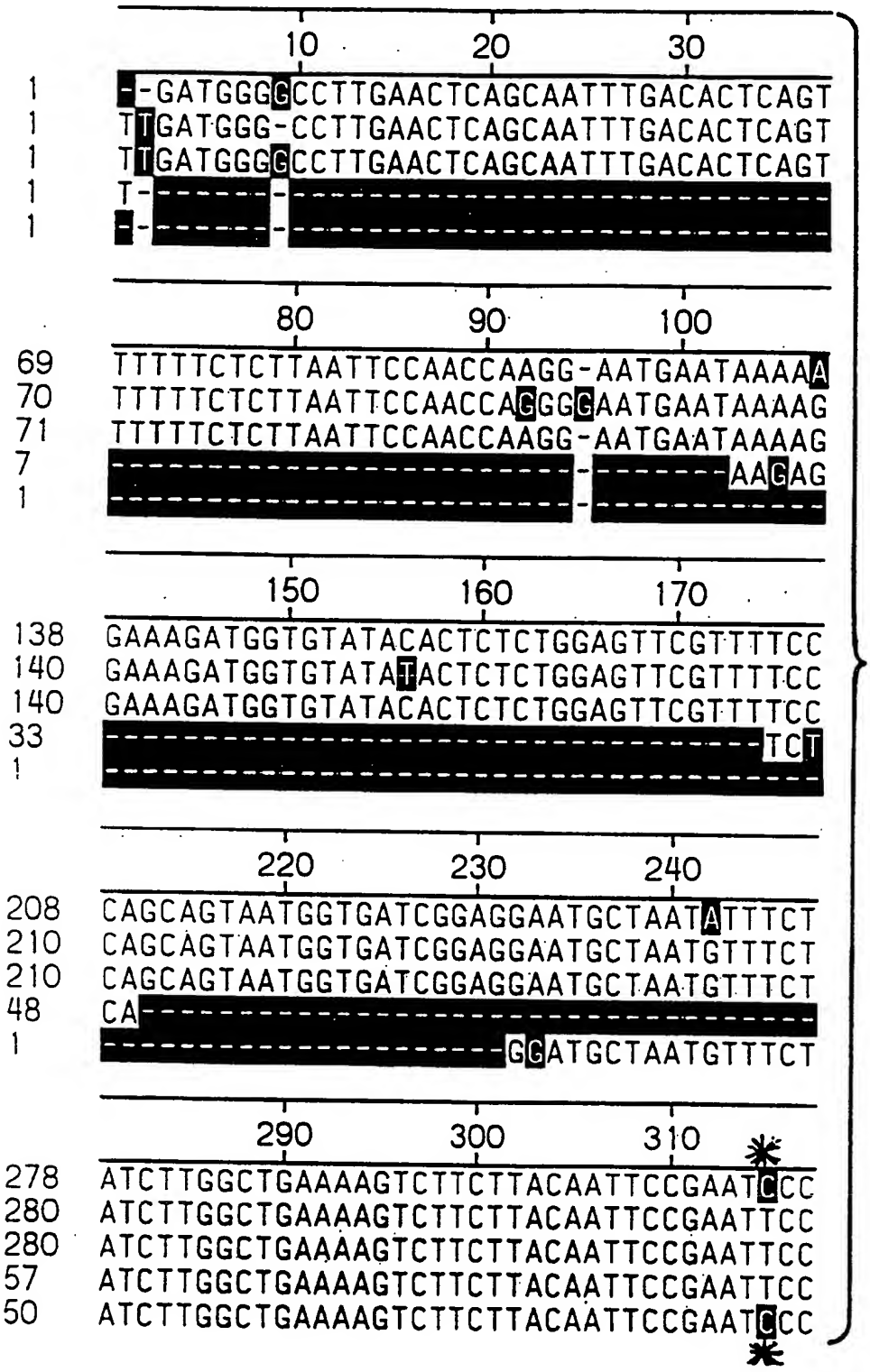

Fig.10 Sheet 2

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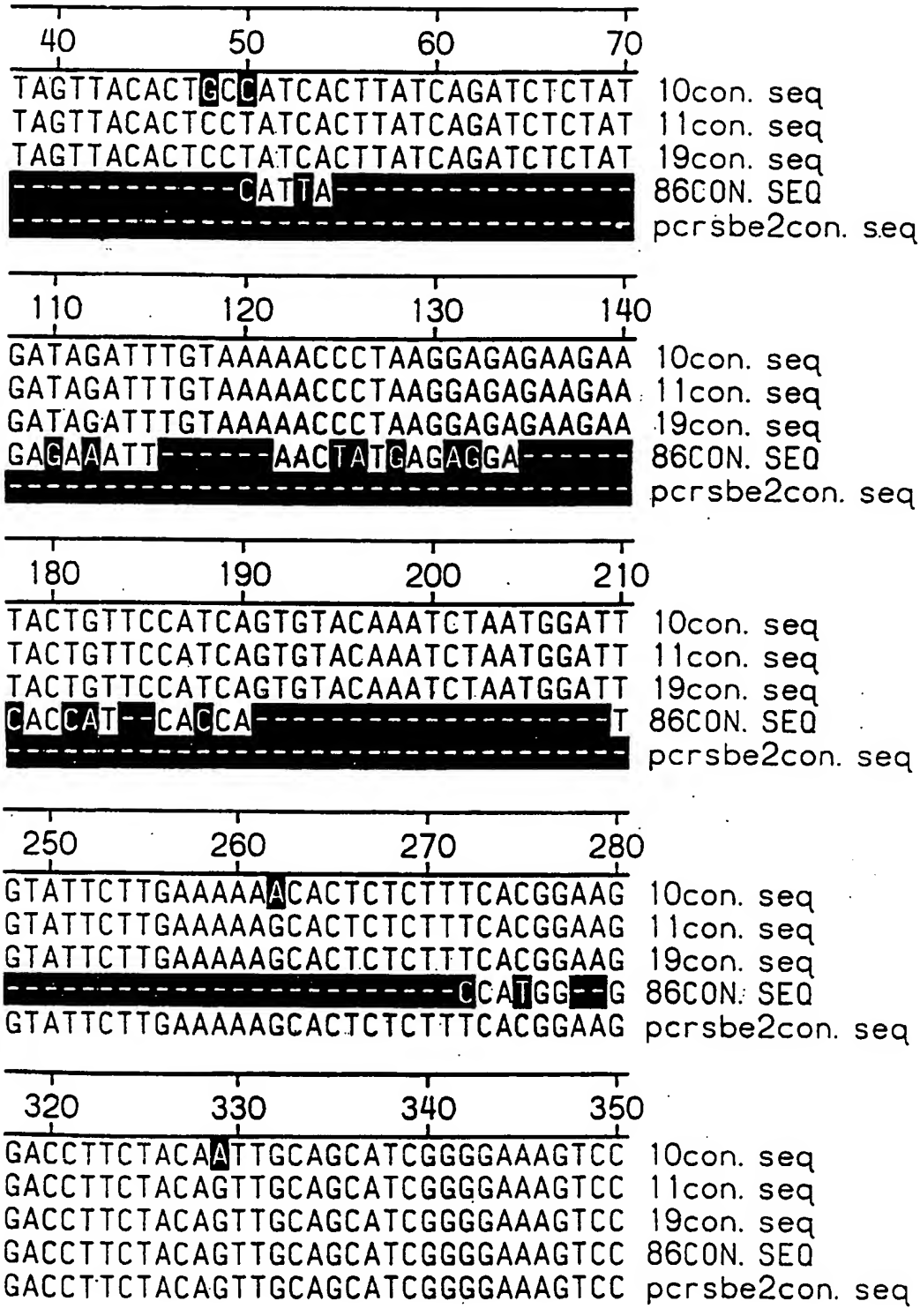

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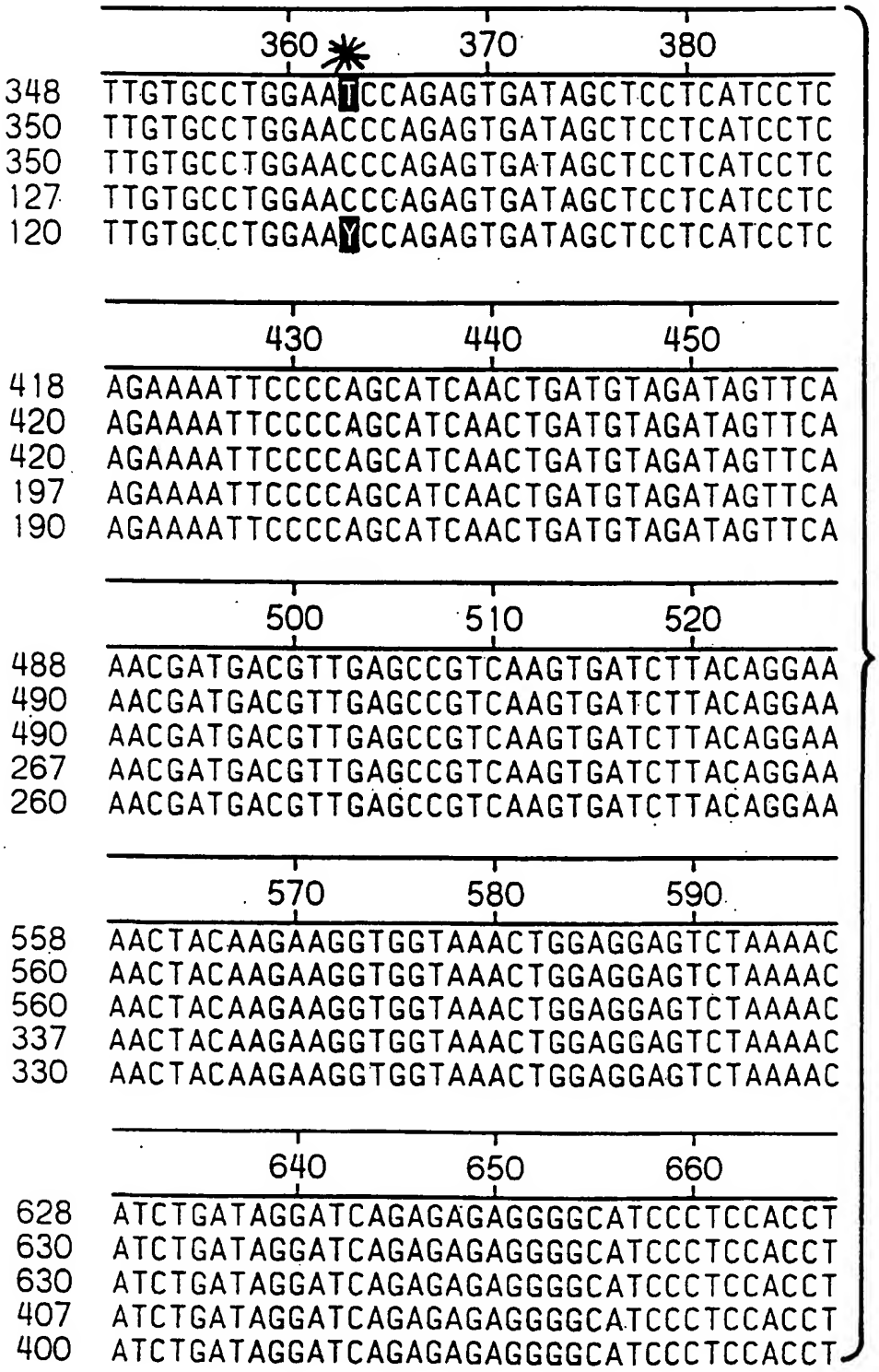

Fig. 10 Sheet 4

Fig. 10 SHEET 3

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AACAGACCAATTTGAGTTCACTGAGACATCTCC  11con. seq
AACAGACCAATTTGAGTTCACTGAGACATCTCC  19con. seq
AACAAACCAATTTGAGTTCACTGAGACATCTCC  86CON. SEQ
AACAGACCAATTTGAGTTCACTGAGACATCTCC  pcrsbe2con. seq

460        470        480        490
ACAATGGAACACGCTAGCCAGATTAAAACTGAG  10con. seq
ACAATGGAACACGCTAGCCAGATTAAAACTGAG  11con. seq
ACAATGGAACACGCTAGCCAGATTAAAACTGAG  19con. seq
ACAATGGAACACGCTAGCCAGATTAAAACTGAG  86CON. SEQ
ACAATGGAACACGCTAGCCAGATTAAAACTGAG  pcrsbe2con. seq

530        540        550        560
GTGTTGAAGAGCTGGATTTTGCTTCATCACTAC  10con. seq
GTGTTGAAGAGCTGGATTTTGCTTCATCACTAC  11con. seq
GTGTTGAAGAGCTGGATTTTGCTTCATCACTAC  19con. seq
GTGTTGAAGAGCTGGATTTTGCTTCATCACTAC  86CON. SEQ
GTGTTGAAGAGCTGGATTTTGCTTCATCACTAC  pcrsbe2con. seq

600        610        620        630
ATTAAATACTTCTGAAGAGACAATTATTGATGA  10con. seq
ATTAAATACTTCTGAAGAGACAATTATTGATGA  11con. seq
ATTAAATACTTCTGAAGAGACAATTATTGATGA  19con. seq
ATTAAATACTTCTGAAGAGACAATTATTGATGA  86CON. SEQ
ATTAAATACTTCTGAAGAGACAATTATTGATGA  pcrsbe2con. seq

670        680        690        700
GGACTTGGTCAGAAGATTTATGAAATAGACCCC  10con. seq
GGACTTGGTCAGAAGATTTATGAAATAGACCCC  11con. seq
GGACTTGGTCAGAAGATTTATGAAATAGACCCC  19con. seq
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Fig.10 SHEET 4

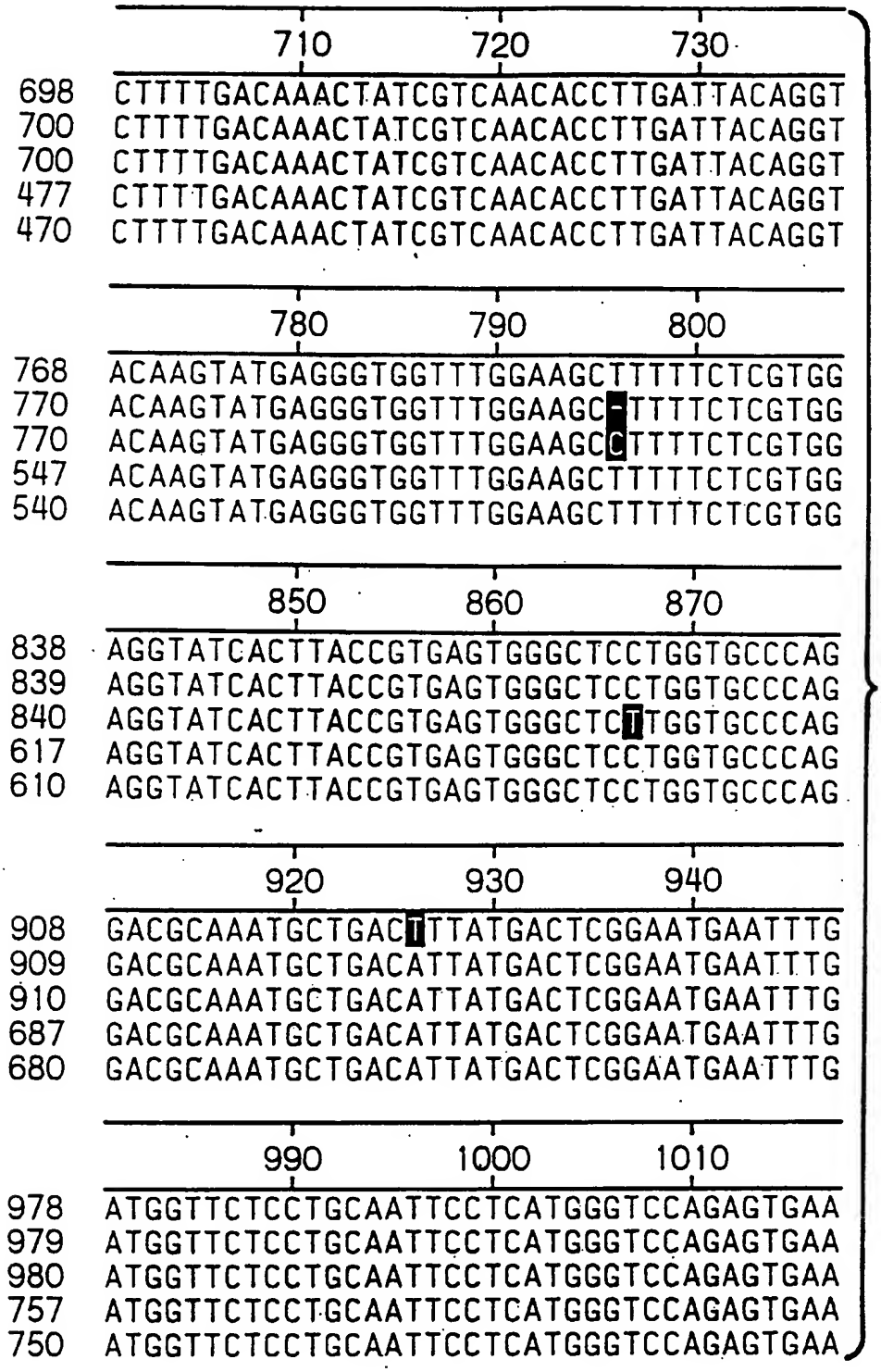


Fig.10 Sheet 6

Fig. 10 SHEET 5

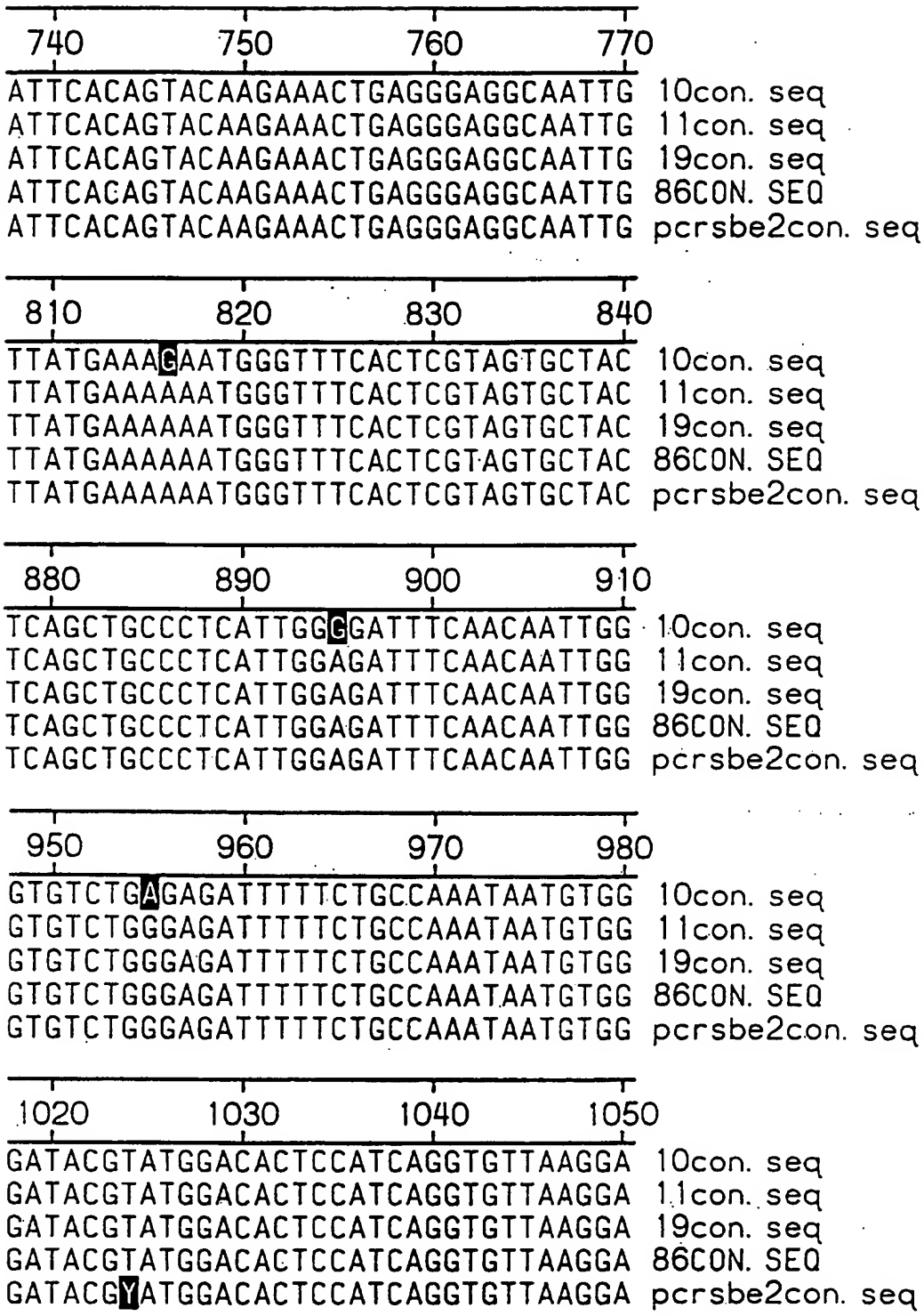


Fig. 10 SHEET 6

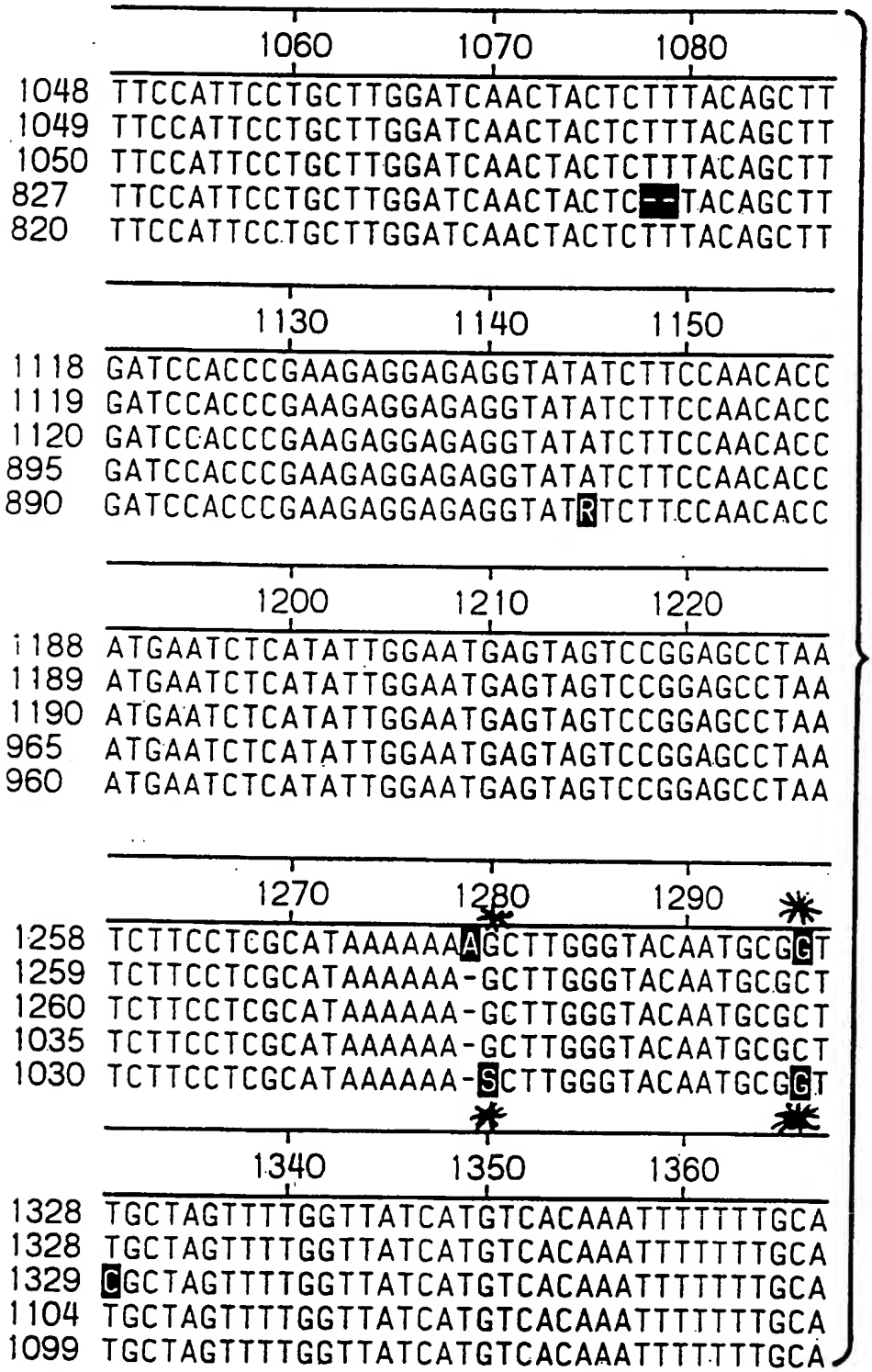
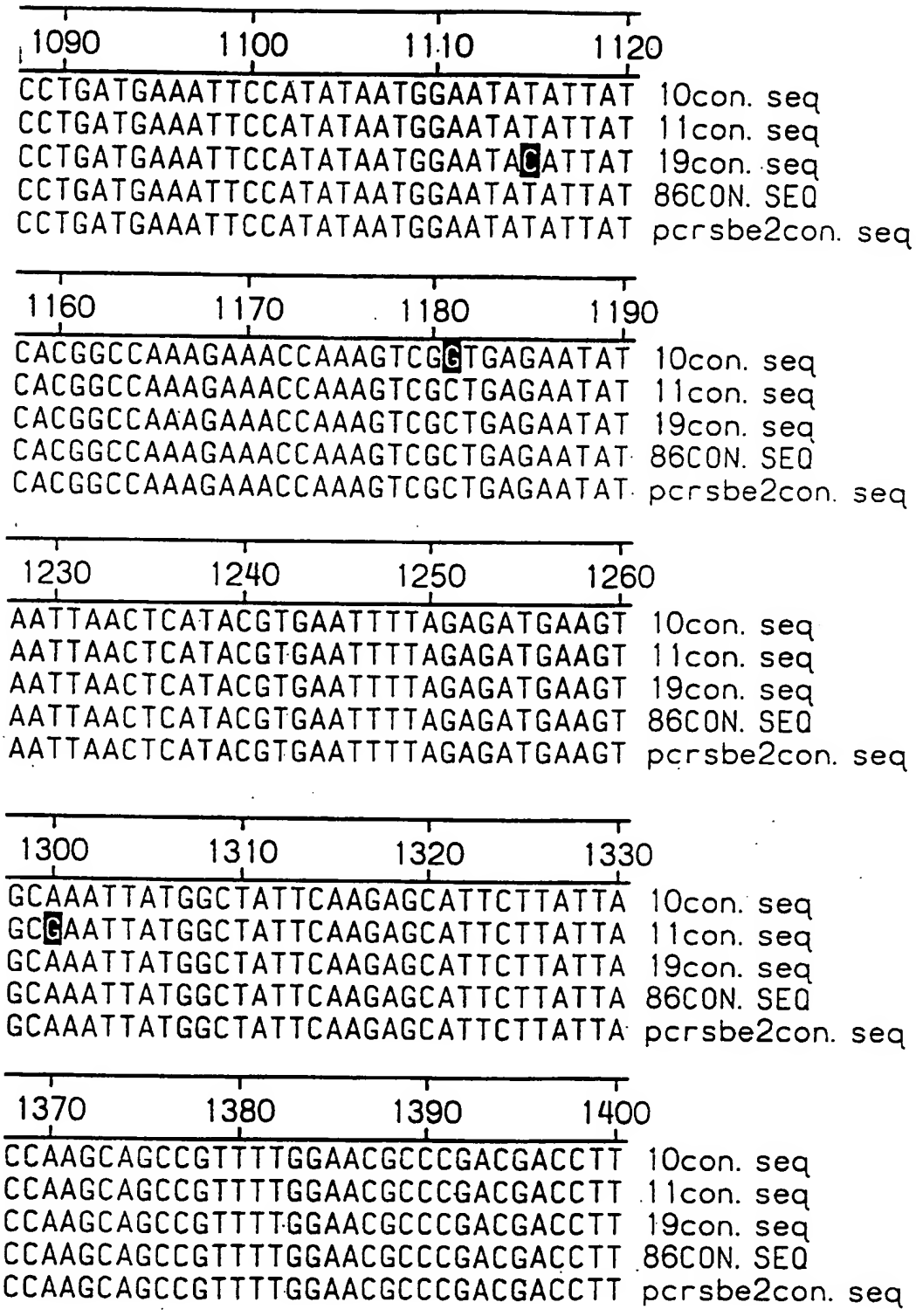


Fig.10 Sheet 8

Fig. 10 SHEET 7



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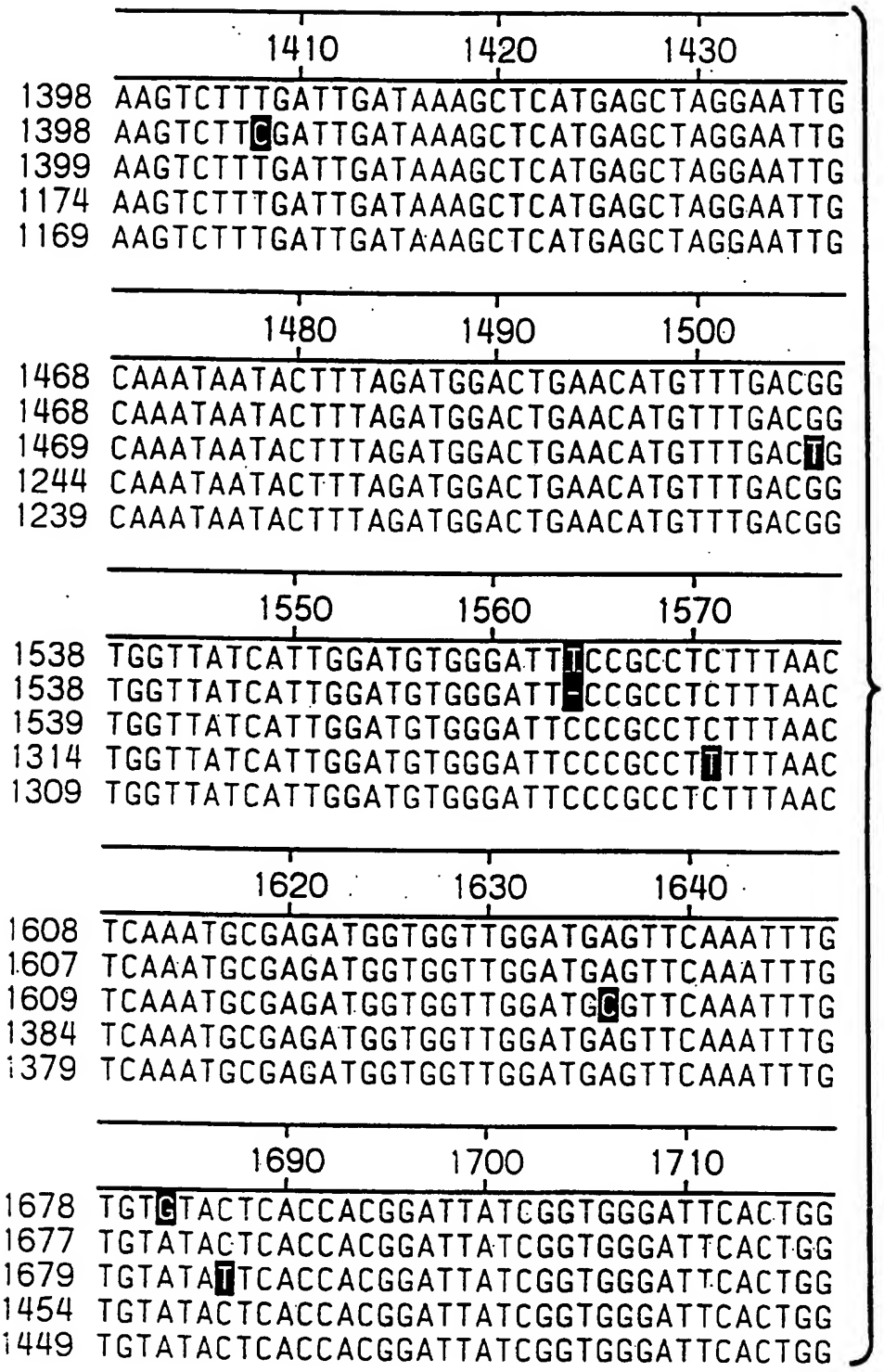
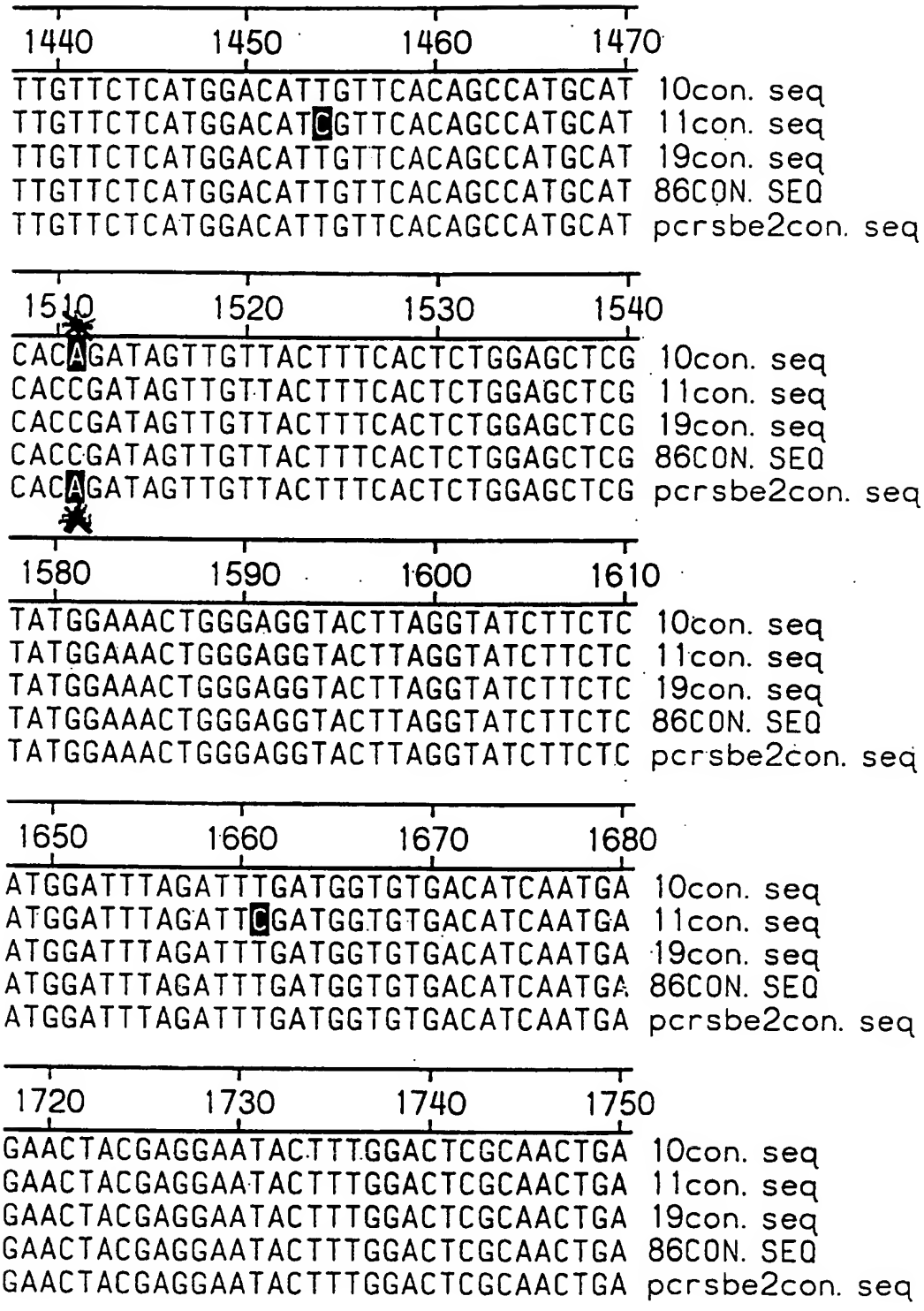


Fig. 10 Sheet 10

Fig. 10 SHEET 9



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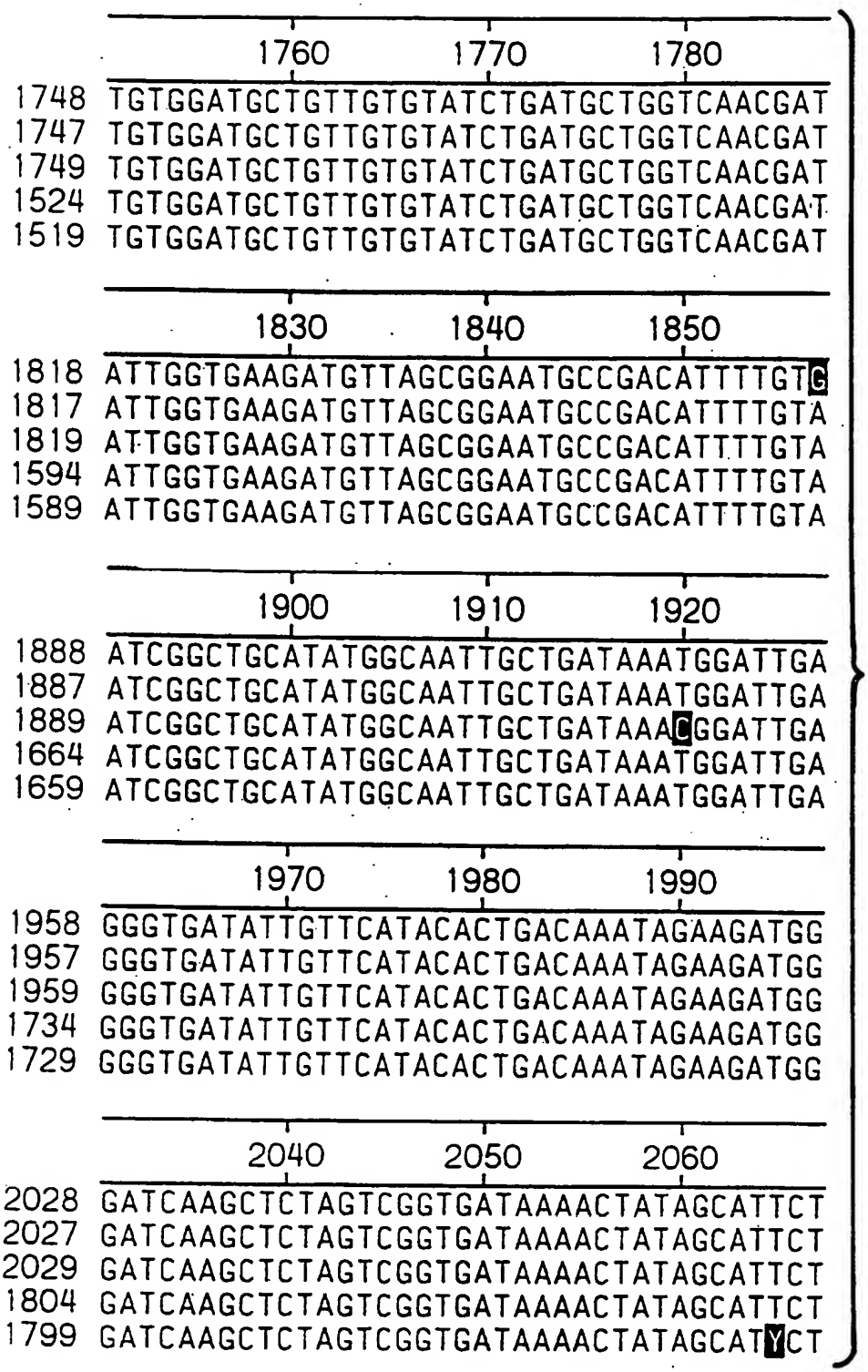

Fig.10 Sheet 12

Fig. 10 SHEET 11

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CTTATTCATGGGCTTTTCCCAGATGCAATTACC 86CON. SEQ
CTTATTCACGGGCTTTTCCCAGATGCAATTACC pcrsbe2con. seq

1860        1870        1880        1890
TTCCCGTTCAAGATGGGGGTGTTGGCTTTGACT 10con. seq
TTCCCGTTCAAGATGGGGGTGTTGGCTTTGACT 11con. seq
TTCCCGTCCAAGAGGGGGGTGTTGGCTTTGACT 19con. seq
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1930        1940        1950        1960
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GTTGCTCAAGAAACGGGATGAGGATTGGAGAGT 11con. seq
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2000        2010        2020        2030
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TCGGAAAAGTGTGTTTCATACGCTGAAAGTCAT 11con. seq
TCGGAAAAGTGTGTTTCATACGCTGAAAGTCAT 19con. seq
TCGGAAAAGTGTGTTTCATACGCTGAAAGTCAT 86CON. SEQ
TCGGAAAAGTGTGTTTCATMCGCTGAAAGTCAT pcrsbe2con. seq

2070        2080        2090        2100
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Fig. 10 SHEET 12

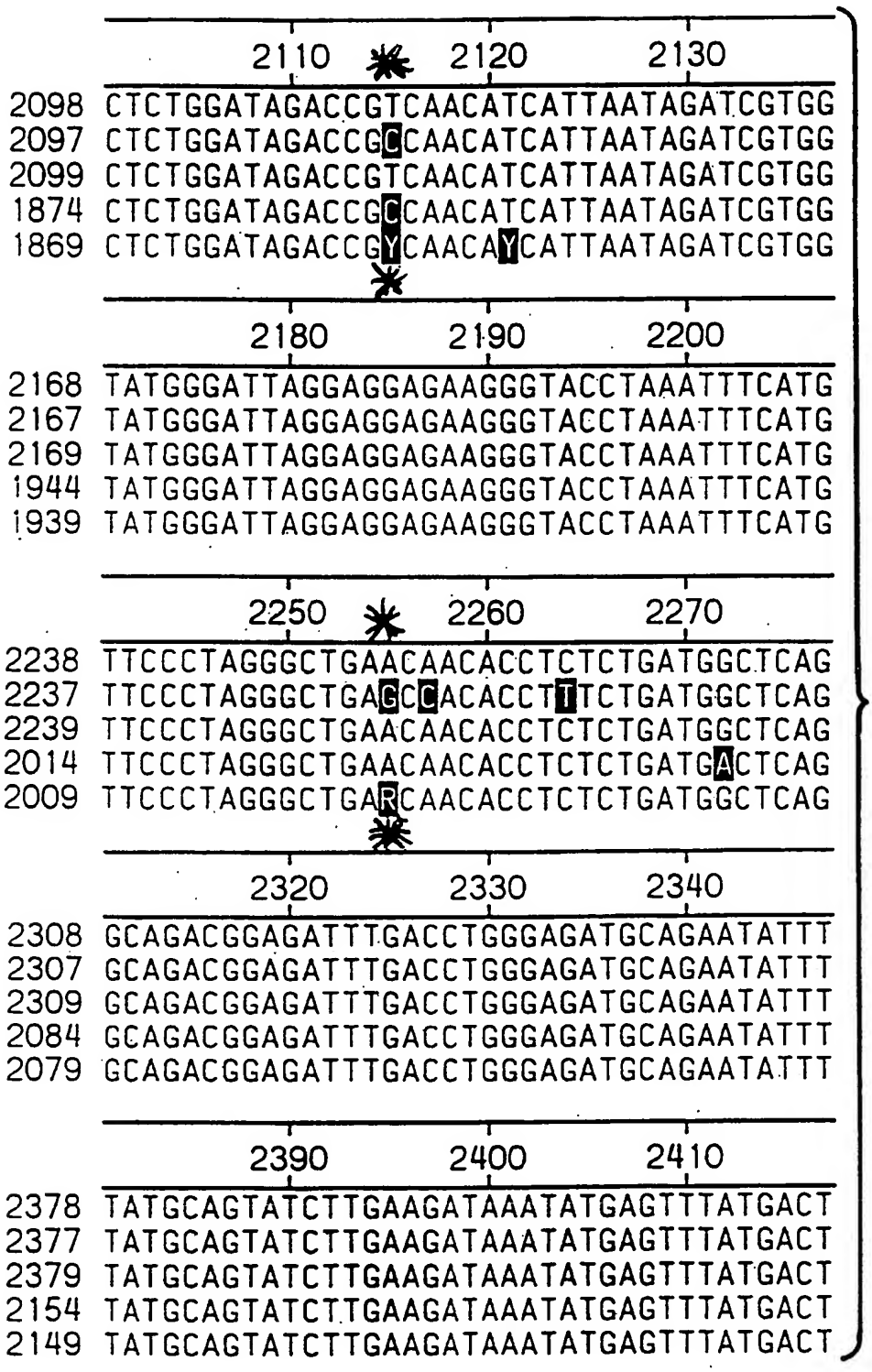

Fig.10 Sheet 14

Fig. 10 SHEET 13

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GATAGCATTGCACAAGATGATTAGGCTTGTAAC 86CON. SEQ
GATAGCATTGCACAAGATGATTAGGCTTGTAAC pcrsbe2con. seq

2210        2220        2230        2240
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GGAAATGAATTCGGCCACCCTGAGTGGATTGAT 11con. seq
GGAAATGAATTCGGCCACCCTGAGTGGATTGAT 19con. seq
GGAAATGAATTCGGCCACCCTGAGTGGATTGAT 86CON. SEQ
GGAAATGAATTCGGCCACCCTGAGTGGATTGAT pcrsbe2con. seq

2280        2290        2300        2310
TAATTCCCAGAAACCAATTCAGTTATGATAAAT 10con. seq
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2350        2360        2370        2380
AAGATACCGTGGGTTGCAAGAATTTGACCGGGC 10con. seq
AAGATACCATGGGTTACAAGAATTTGACTGGGC 11con. seq
AAGATACCGTGGGTTGCAAGAATTTGACCGGCC 19con. seq
AAGATACCGTGGGTTGCAAGAATTTGACCGGGC 86CON. SEQ
AAGATACCATGGGTTGCAAGAATTTGACCGGGC pcrsbe2con. seq

2420        2430        2440        2450
TCAGAACACCAGTTCATATCACGAAAGGATGAA 10con. seq
TCAGAACACCAGTTCATATCACGAAAGGATGAA 11con. seq
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Fig. 10 SHEET 14

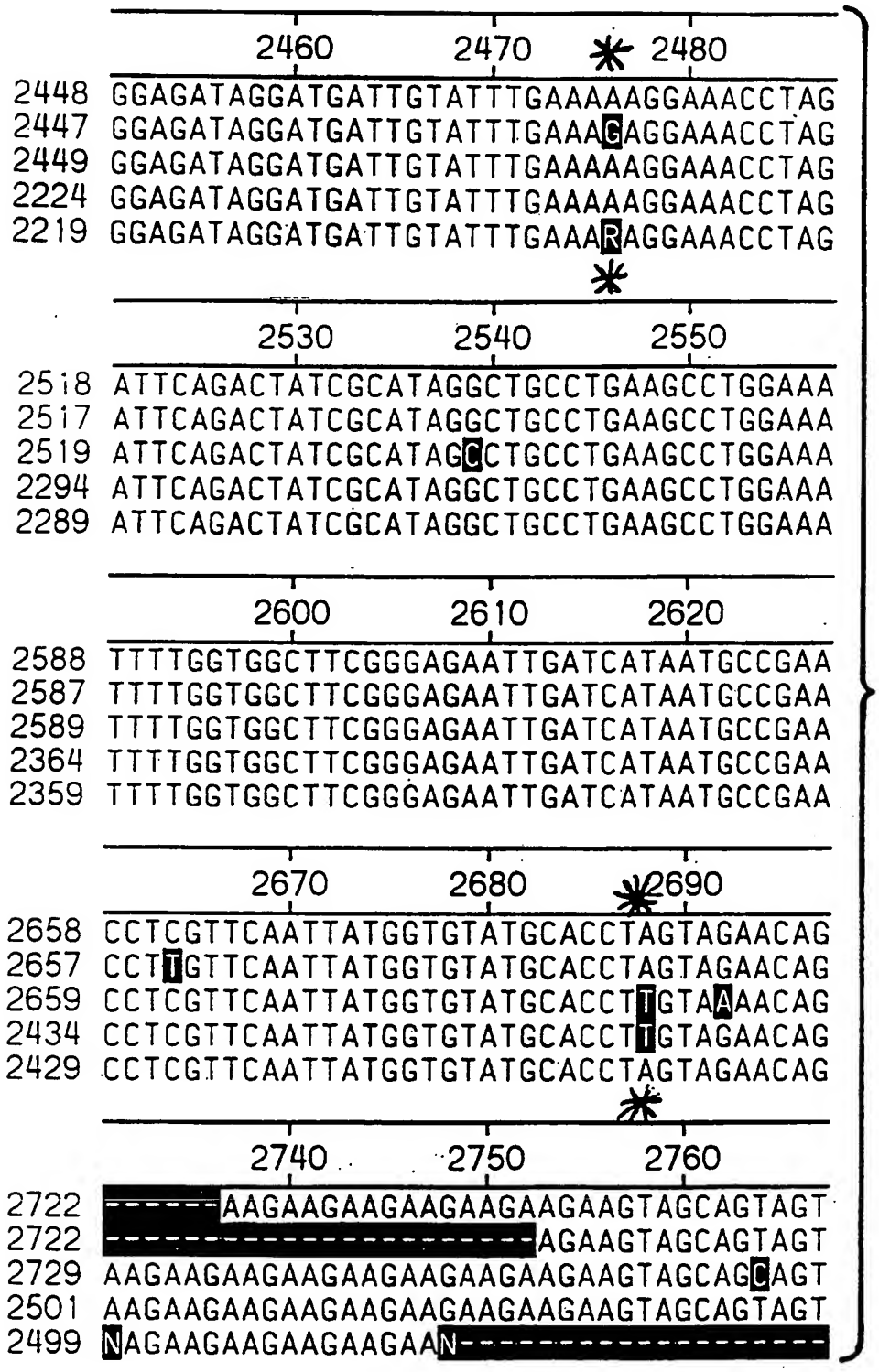
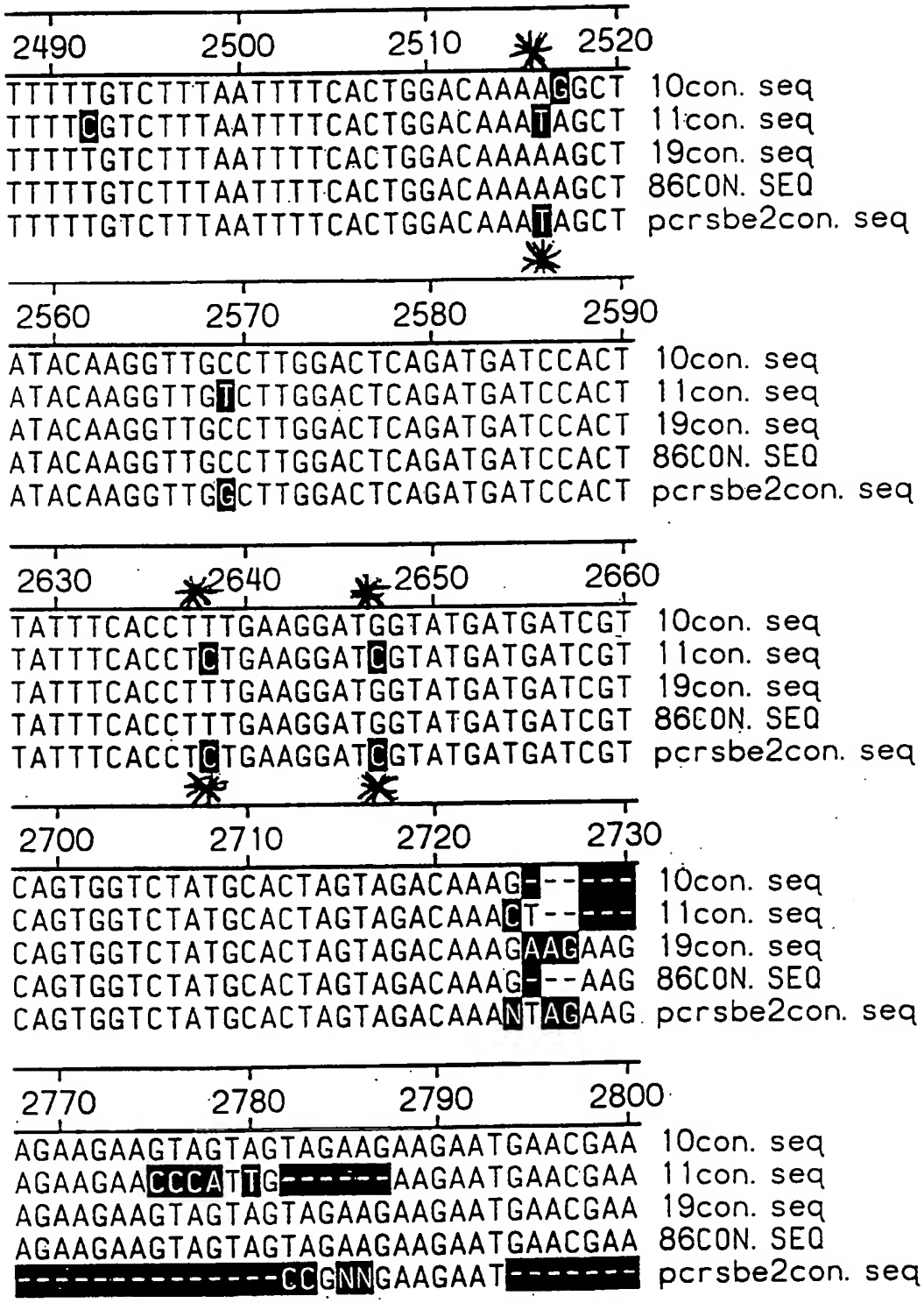


Fig. 10 Sheet 16

Fig. 10 SHEET 15



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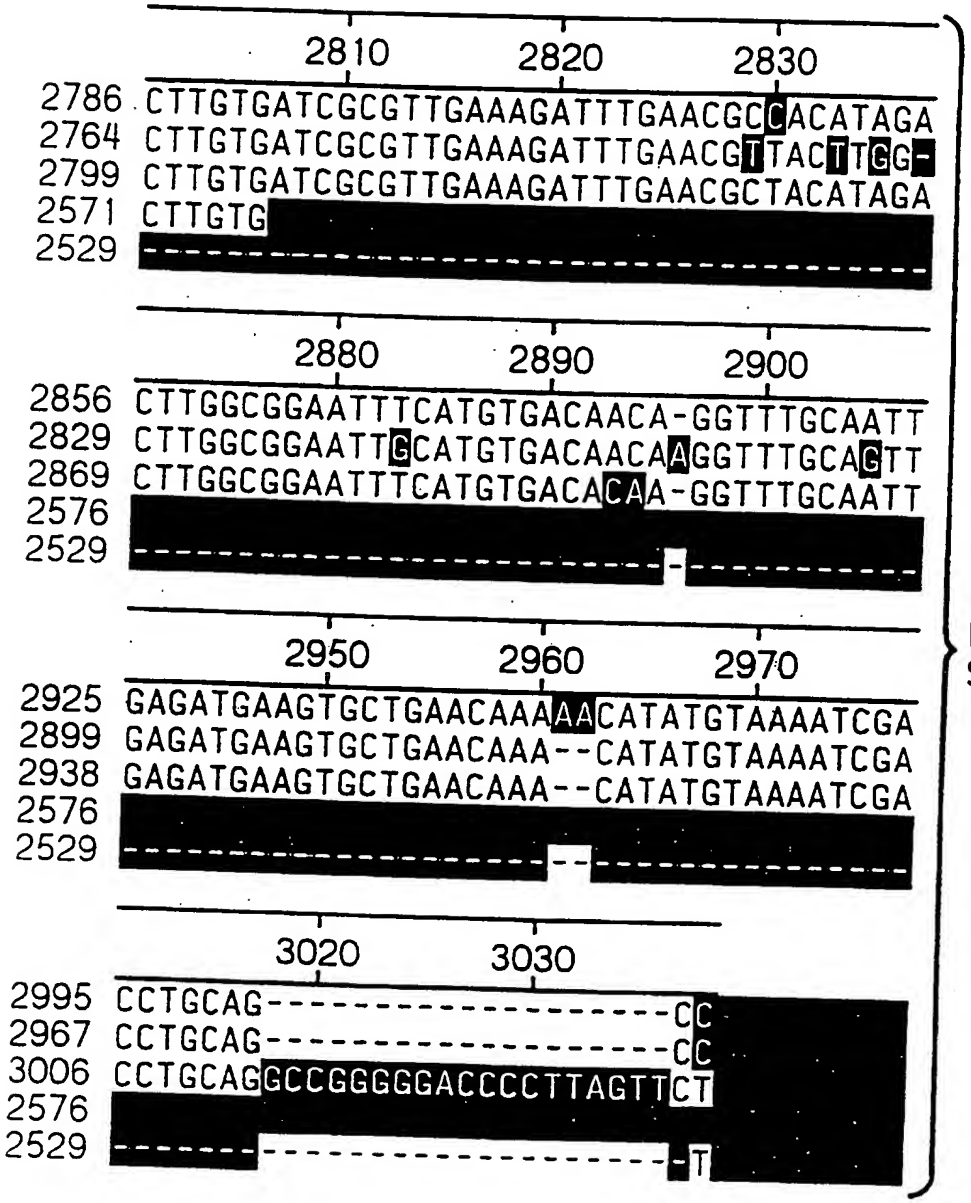

Fig. 10 Sheet 18

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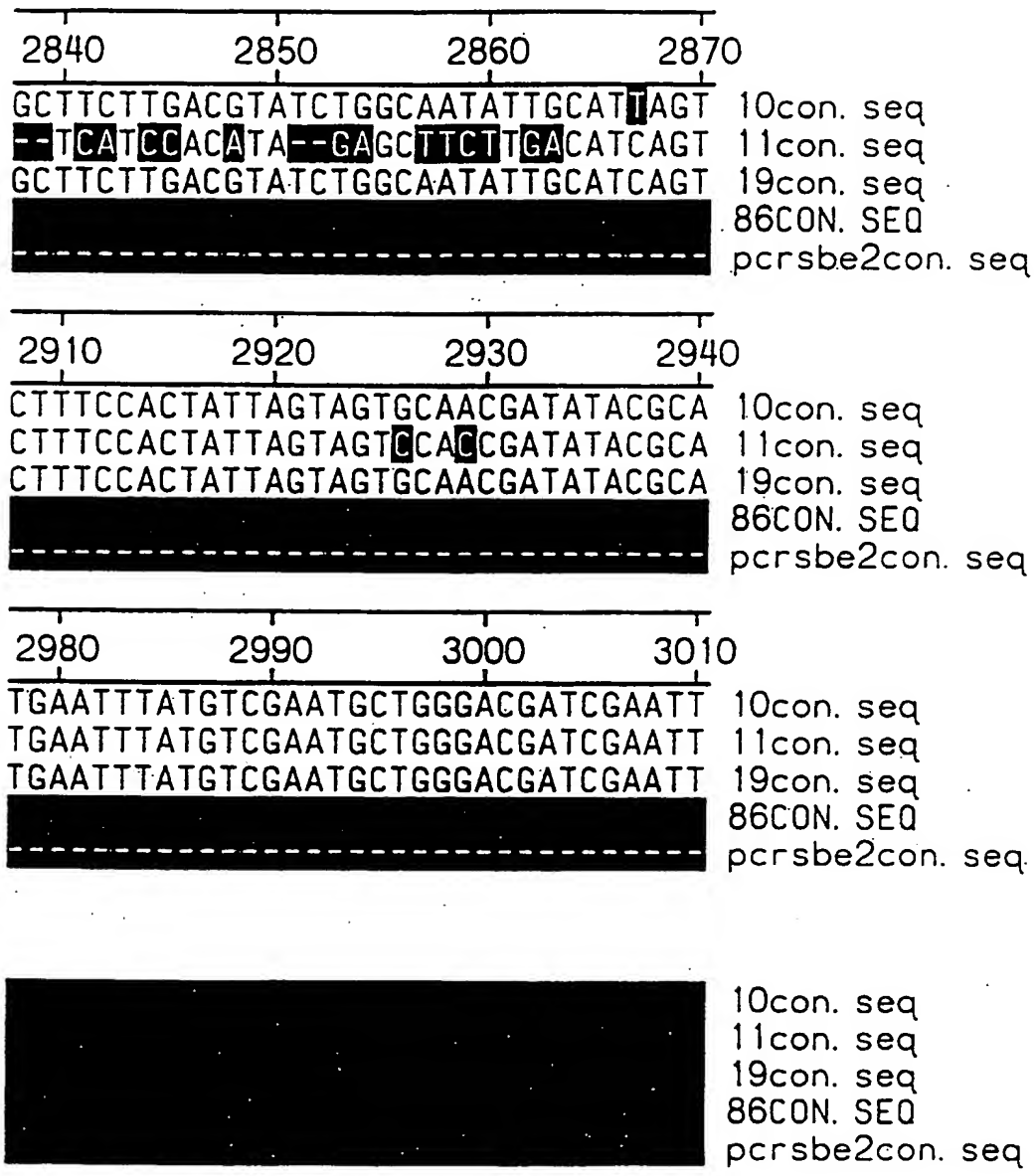

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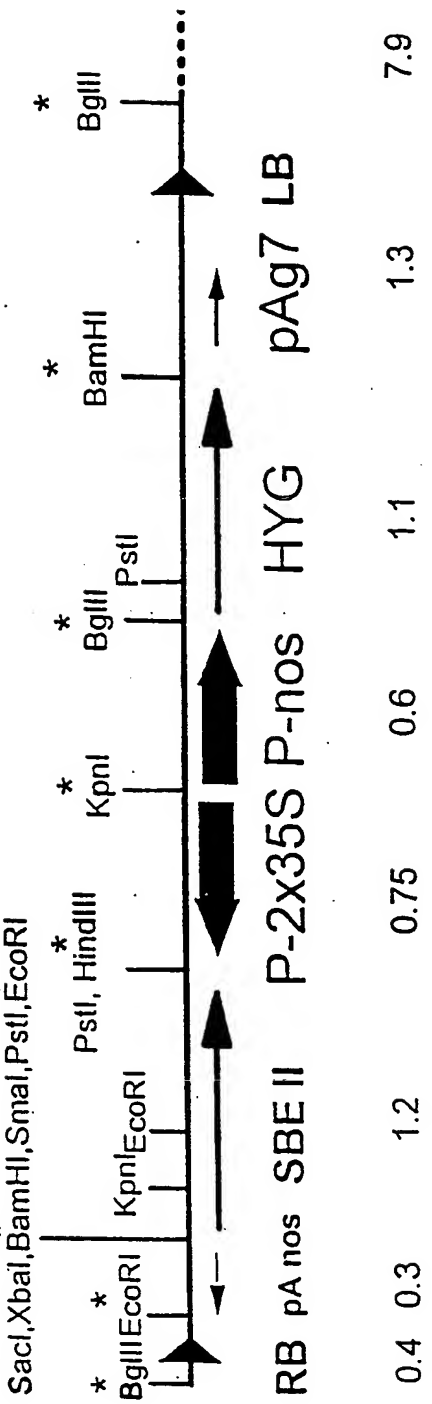
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BamHI
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Fig. 11

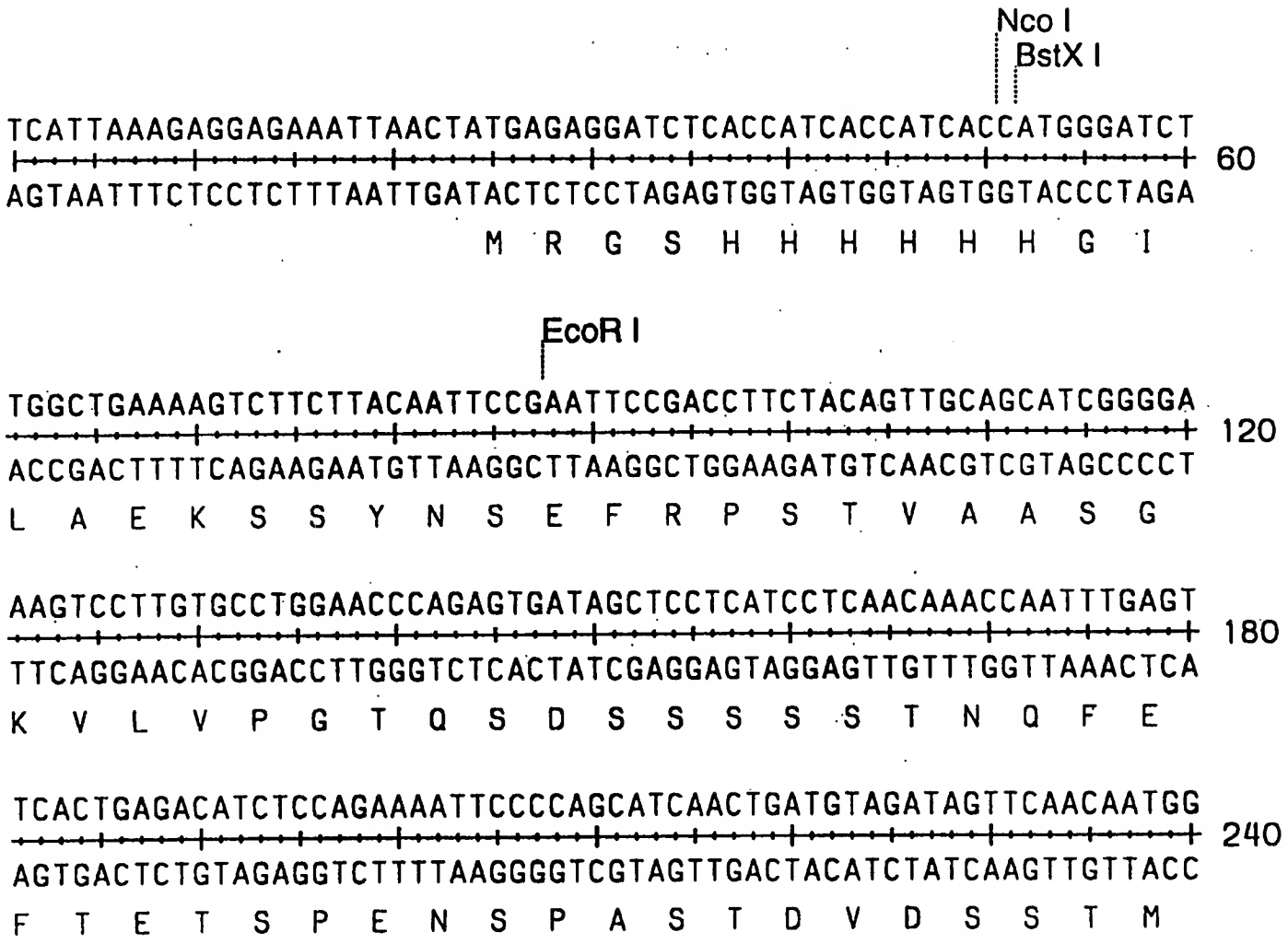

Fig. 12
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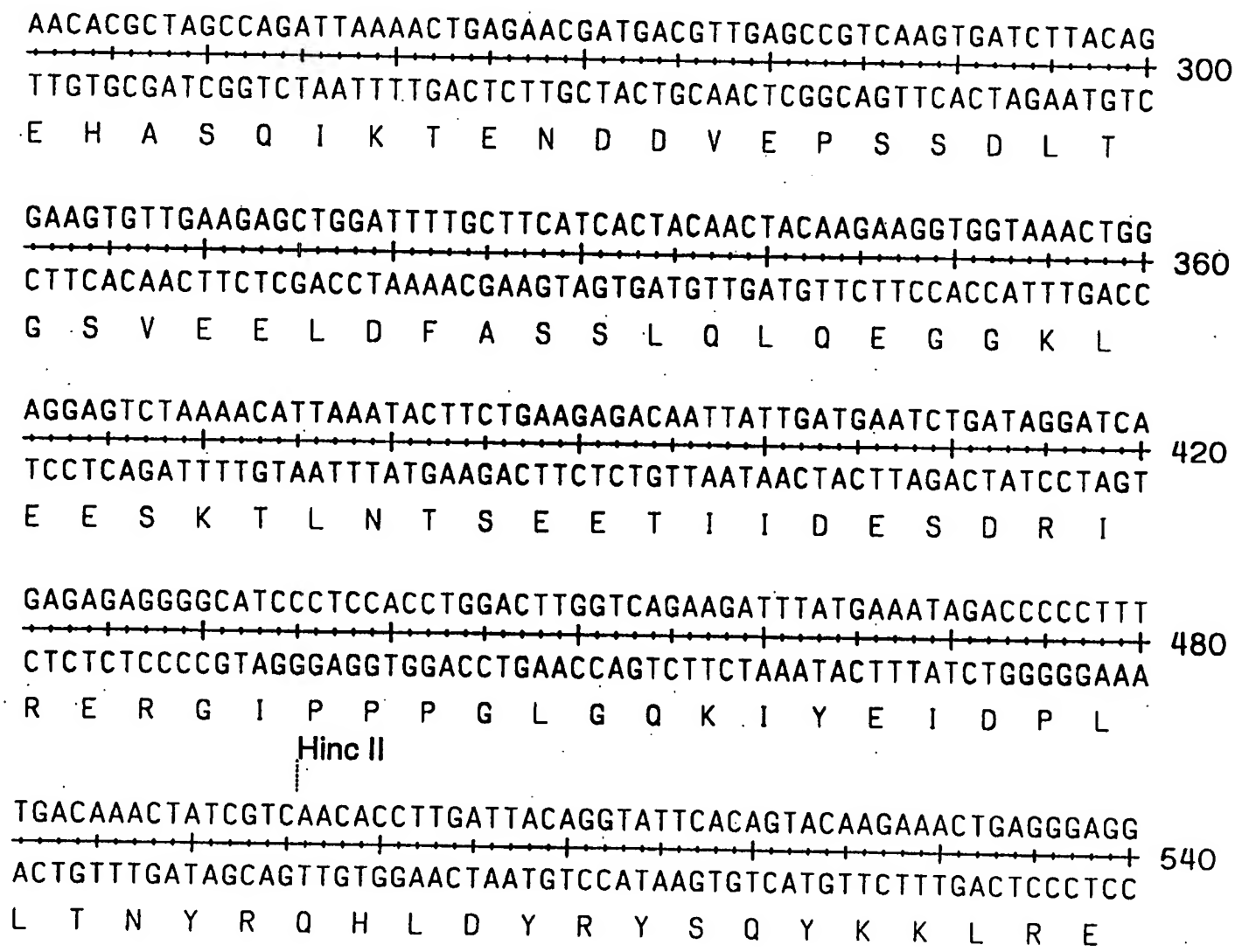

Fig. 12

SHEET 2

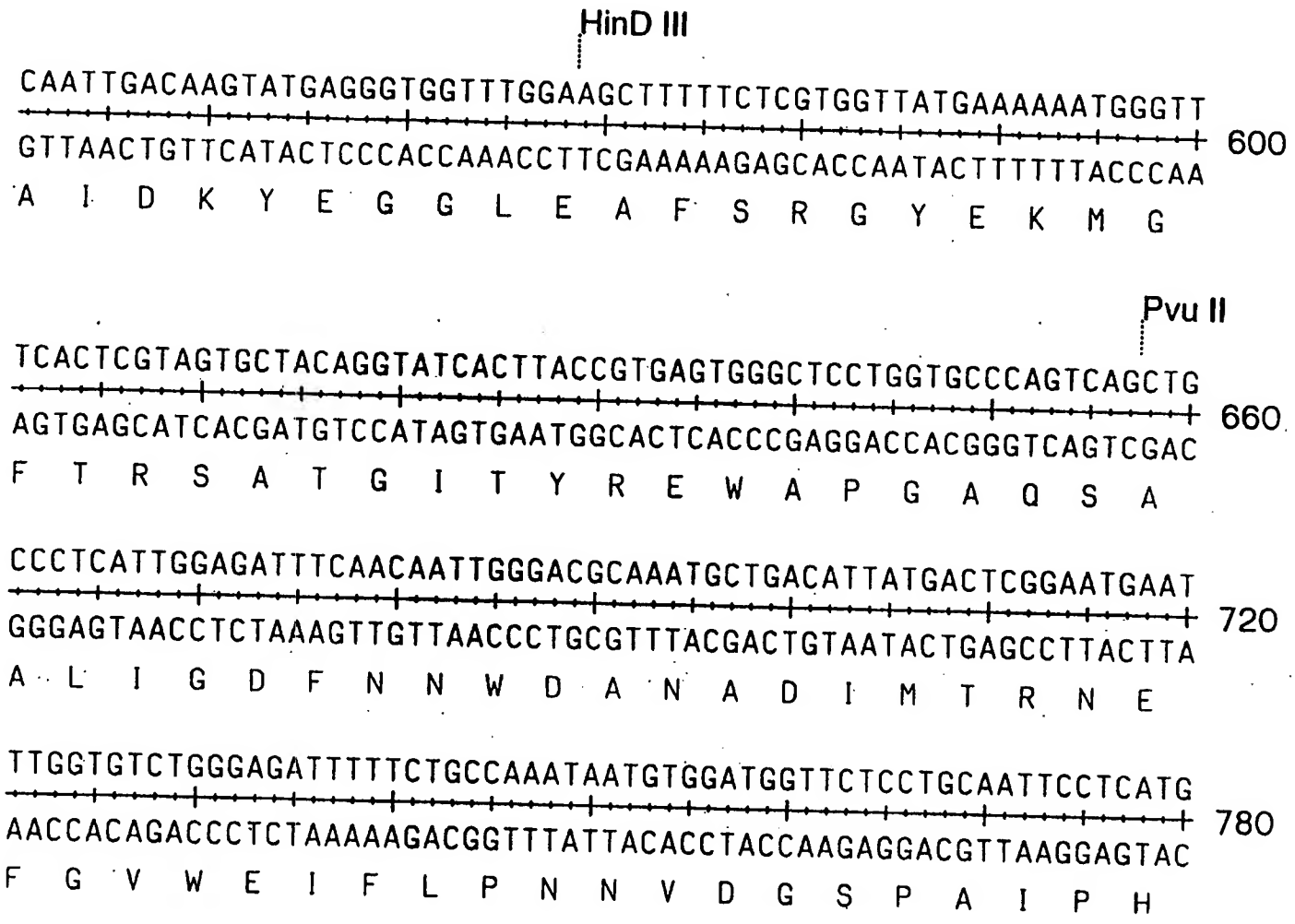

Fig 12
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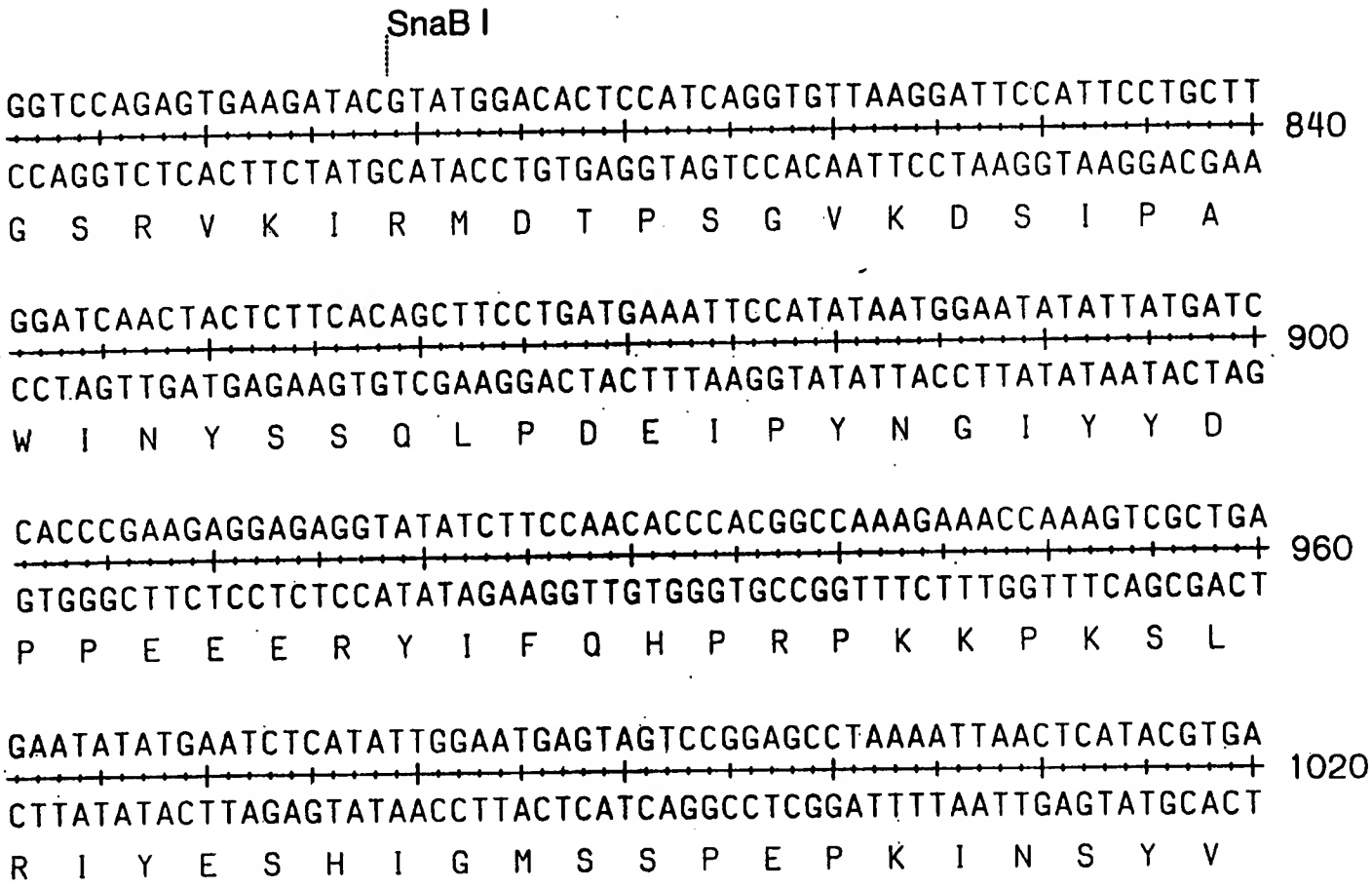

Fig. 12
SHEET 4

Xmn I HinD III

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N F R D E V L P R I K K L G Y N A V Q I

TGGCTATTCAAGAGCATTCTTATTATGCTAGTTTTGGTTATCATGTCACAAATTTTTTG 1140
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M A I Q E H S Y Y Y A S F G G Y H V T T N F F F

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A P S S R F G G T P D D L K S L I D K A H

Nsi I

AGCTAGGAATTGTTGTTCTCATGGACATTGTTCACAGCCATGCATCAAATAATACTTTAG 1260
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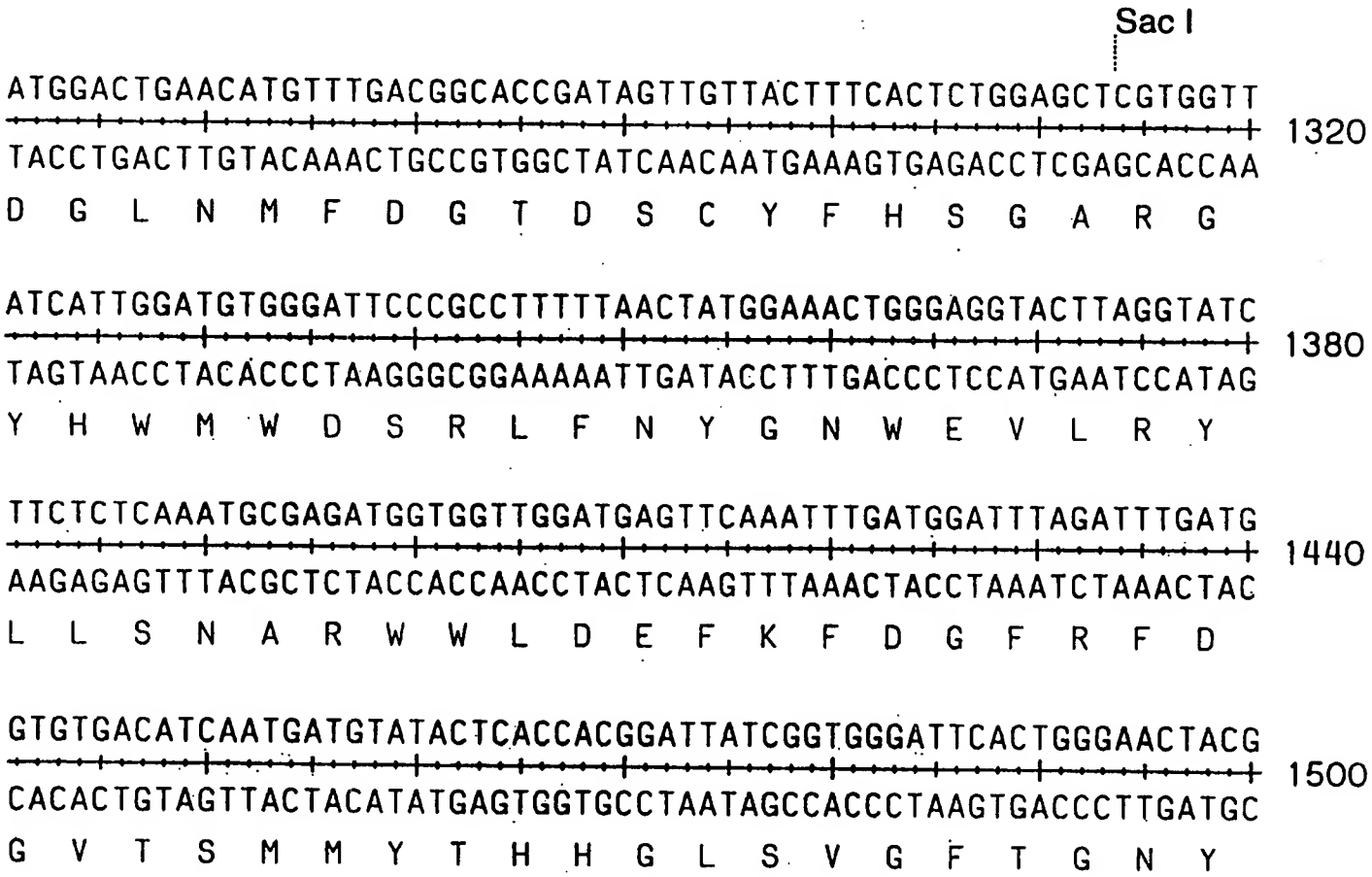
Fig.12
SHEET 5



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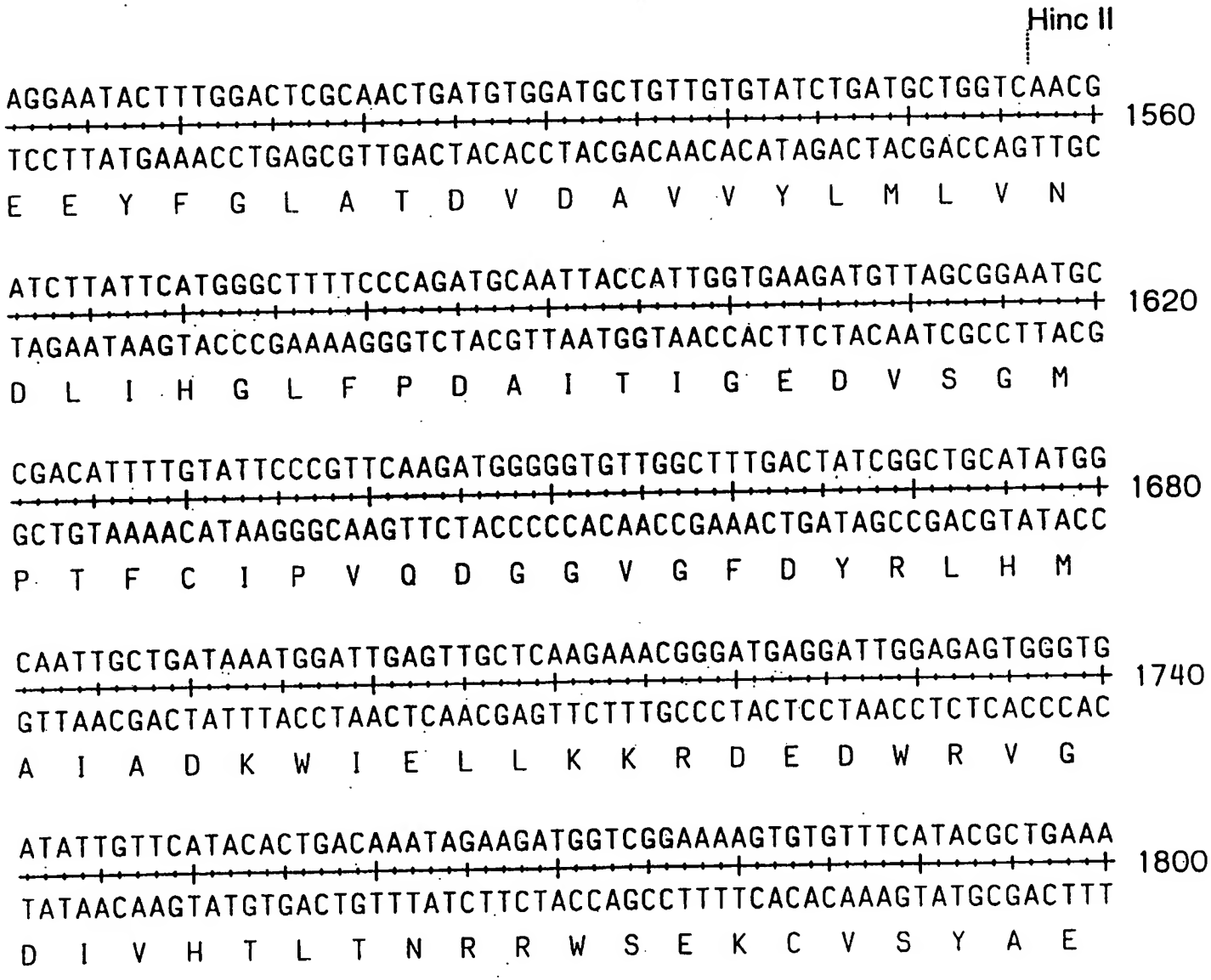

Fig 12
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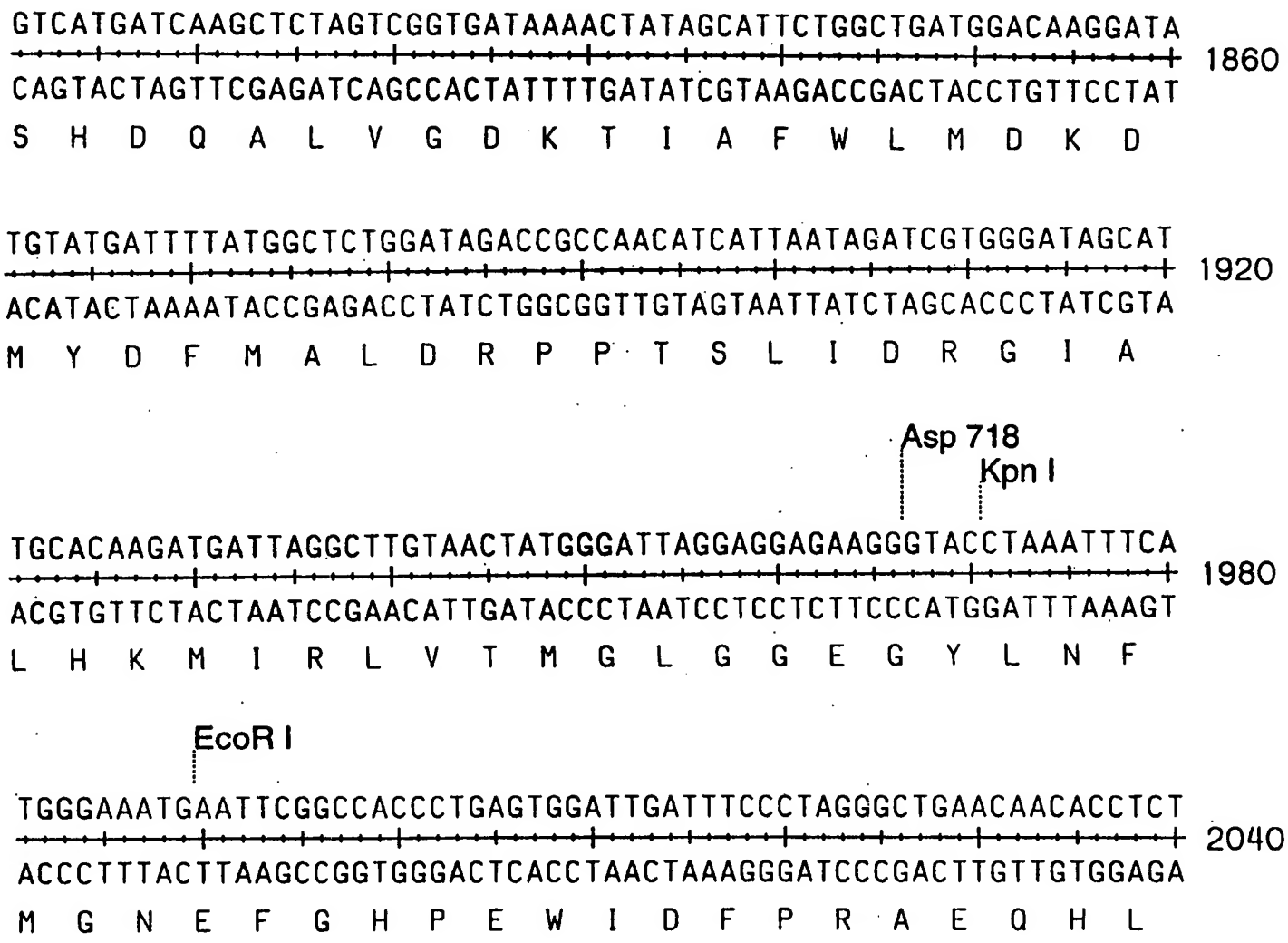

Fig 12
SHEET 8

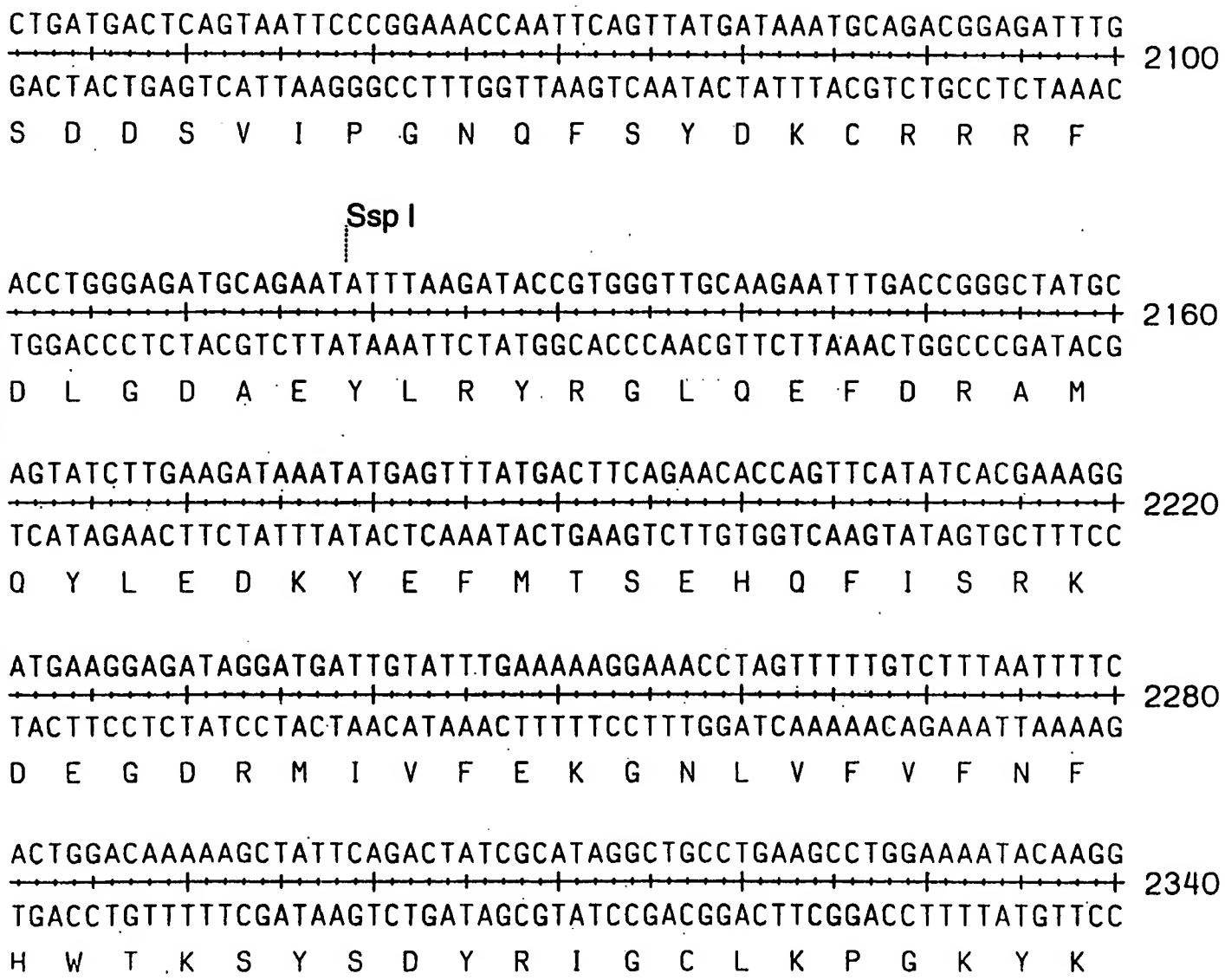

Fig. 12
SHEET 9

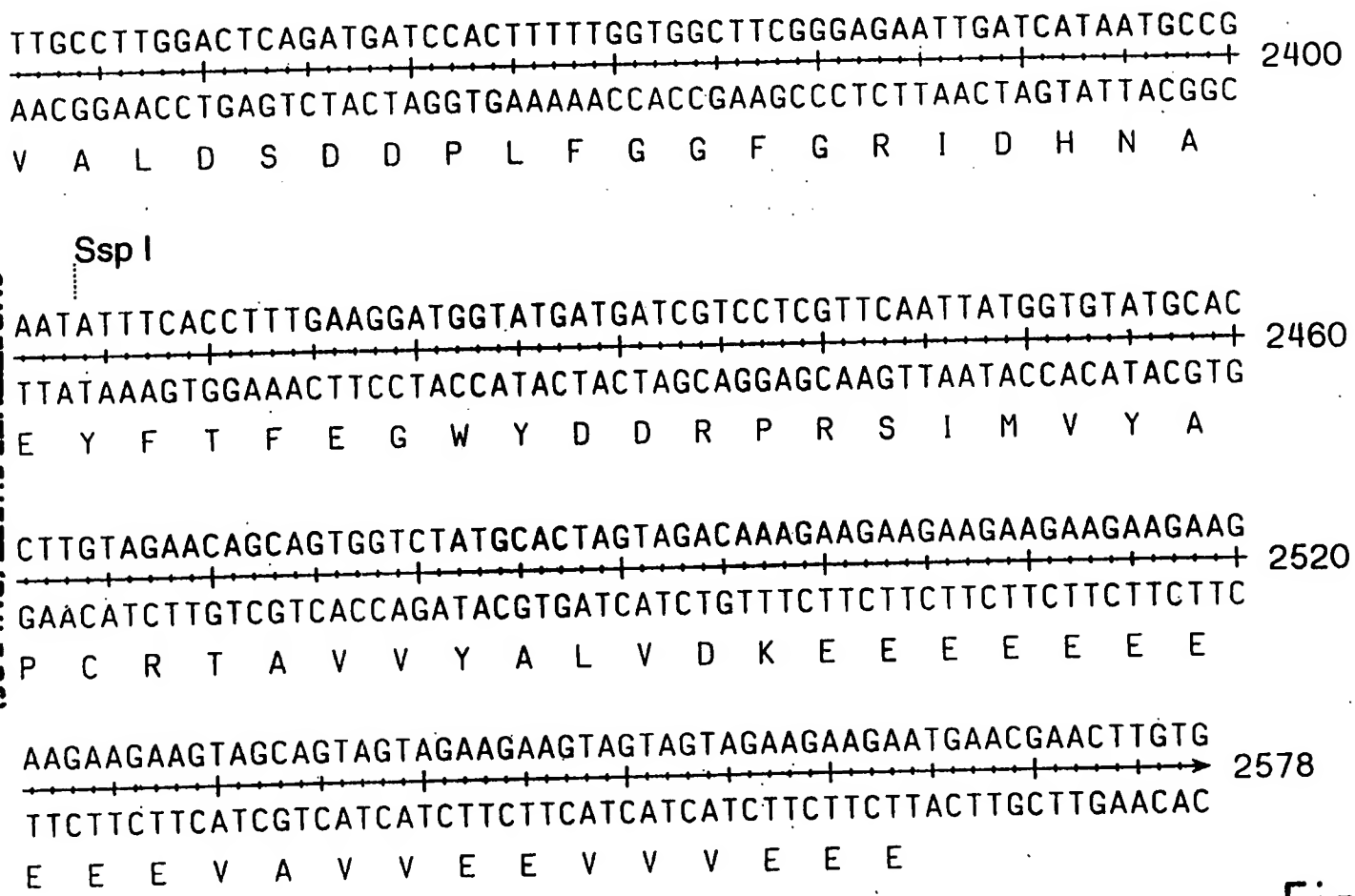

Fig 12
SHEET 10

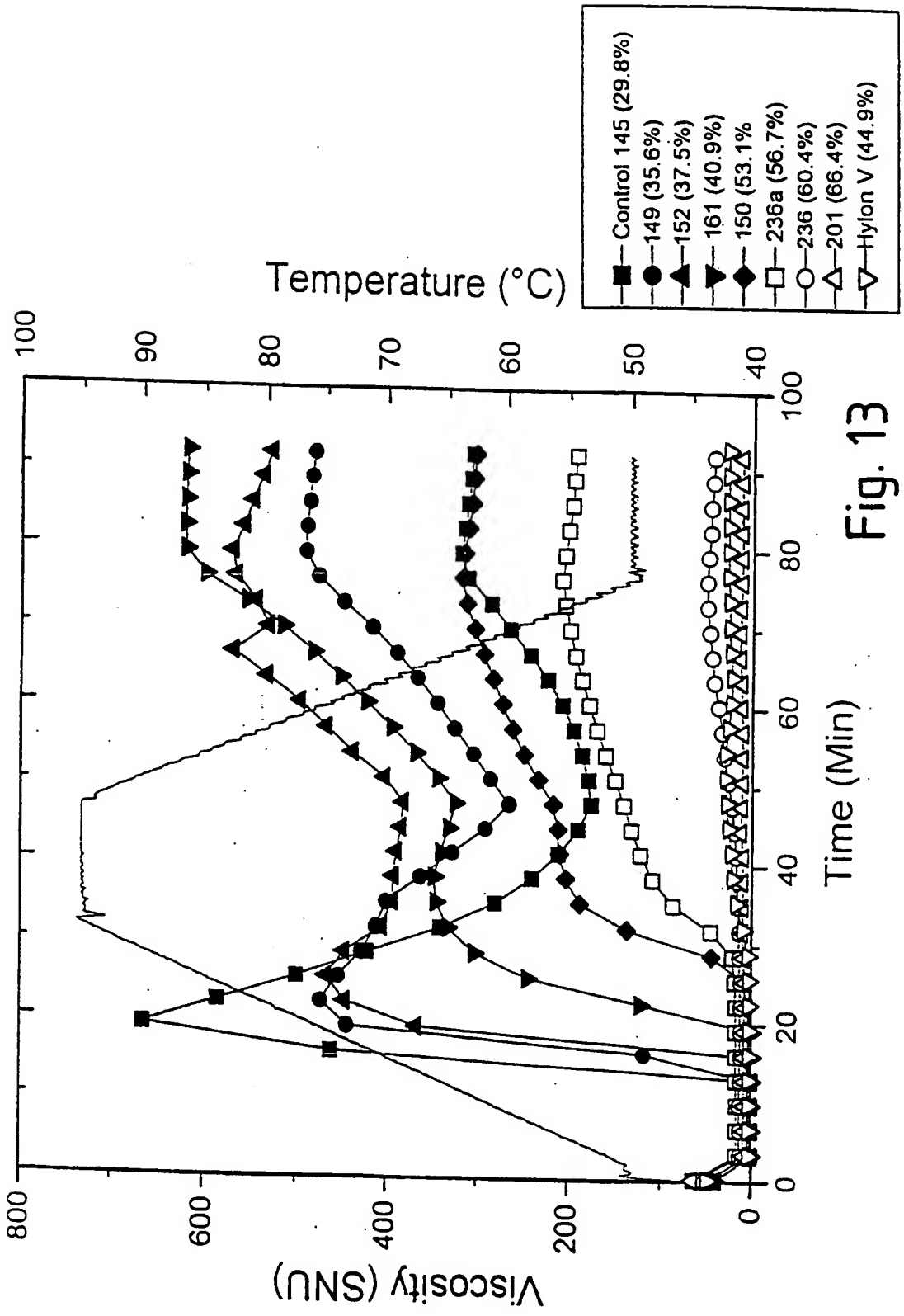
Temperature (°C)
100
90
80
70
60
50
40
Viscosity (SNU)
800
600
400
200
0
Time (Min)
0
20
40
60
80
100
Control 145 (29.8%)
149 (35.6%)
152 (37.5%)
161 (40.9%)
150 (53.1%
236a (56.7%)
236 (60.4%)
201 (66.4%)
Hylon V (44.9%)

Fig. 13